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Å see Aa
A see Ae
Ö see Oe
Ø see Oe

st[illegible] at −20°C apparently does not affect se[illegible] and TG values (37 38)

Assay methods Serum total cholesterol (9) and serum TG (3) were determined using Technicon AutoAnalyzer equipment Three consecutive lots of the Helsinki Control Circle frozen (not lyophilized) human control serum were used for calibration The serum was regularly analysed for cholesterol by 20 other laboratories and for TG by 7 other laboratories Originally the means of the cholesterol and TG concentrations obtained by the other laboratories for one lot of the control serum were taken as being correct Subsequently new lots were assigned cholesterol and TG concentrations by comparing them with the preceding one and with other control sera with a view to maintain the original level of accuracy (original degree of bias)

Units Total serum cholesterol and TG concentrations will usually be given in mg/100 ml and in some cases also in mmol/l TG concentrations were converted to molar units using the molecular weight 885 (triolein)

Effects of the glucose load To study the possible effect of the glucose load on the serum TG concentration (39) plasma samples taken immediately before and 1 hour after the glucose load were obtained from 148 Finns who had fasted 12 hours or longer The mean TG concentration of the initial samples was 107 96 mg/100 ml (range 23 9–318 2) and the mean of the 1 hour samples 105 50 (range 24 2–294 1) The correlation coefficient between the two sets of TG results was 0 973

The method of least squares (24) was used to estimate the parameters of the linear functional model describing the relationship between the true initial TG concentration (x) and the true 1 hour concentration (y) The model obtained was $y = 0\ 978\ x$ Thus the 1 hour values were estimated to be only 2% lower than the initial values This does not affect the validity of the results

Quality control Every 20th serum analysed was a control serum During the study our laboratory participated in the quality control of the Helsinki Control Circle The monthly means of our cholesterol and TG determinations on the control sera of the Circle differed 1–3% from the means calculated for all participating laboratories The laboratory has also participated in the Cooperative Cholesterol and Triglyceride Standardization Programs organized jointly by the Center for Disease Control (Atlanta Georgia USA) and the WHO Within the limits set by the programs our laboratory s accuracy and precision were acceptable for all the serum pools analysed with the exception of one cholesterol serum pool for which we obtained too high a concentration (the Center for Disease Control had a mean 6 7% lower than ours) For all the three cholesterol serum pools the Center for Disease Control had a value on an average 5 2% lower than ours

Of all the serum samples 3% were reanalysed on the following working day In the meantime these sera had been stored at +4 C Calculated from these duplicate samples the S D for cholesterol assays during the study period varied between 5 5 and 10 5 mg/100 ml (0 14–0 27 mmol/l) and the S D for TG assays between 5 6 and 7 3 mg/100 ml (0 063–0 082 mmol/l) As an exception TG results obtained very early in the study were of a poorer quality with an S D of 21 mg/100 ml (0 237 mmol/l)

Statistical methods Cholesterol and in particular TG frequency distributions are positively skewed (6 8 34) TG distributions can be brought nearer to normal with a logarithmic transformation and in the statistical analyses we generally use log(TG) values Antilogarithms of log (TG) averages are frequently given These are closely similar to the medians of the original distribution

Due to the large number of observations the skewness of the cholesterol distributions was considered not to interfere with statistical interpretations

The statistic g_1 was used as a measure of skewness (10) Its approximate standard error was calculated as suggested by Cramer (10)

Generally when testing differences between age and sex groups the usual t test was used However when testing differences between means of various age groups of Lapp children we used a statistic which has approximately a t-distribution if the distributions are approximately normal (12) although the number of observations in the youngest age group was very small

RESULTS

Effects of length of fast Only small and inconsistent differences were found between the serum cholesterol means of those with the shorter and the longer fast On the other hand the length of the fast

Fig 2 Cholesterol means and the antilogarithm of log (TG) means as a function of age

Table II *Number of persons studied by sex age length of fast and ethnic group*

Age (y)	Fast≤12h Finns	Fast≤12h Lapps	Fast >12 h Finns	Fast >12 h Lapps	All Finns	All Lapps
Males						
0– 4		1		9		10
5– 9		2		33		35
10–14		1		56		57
15–19	439	2	189	50	628	52
20–29	980	6	328	35	1 308	41
30–39	1 137	1	386	41	1 523	42
40–49	1 140	3	412	38	1 552	41
50–59	774	5	321	34	1 095	39
60–69	367	4	235	54	602	58
70–	131	1	90	6	221	7
All	4 968	26	1 961	356	6 929	382
Females						
0– 4		2		14		16
5– 9				29		29
10–14		4		54		58
15–19	370	3	150	49	520	52
20–29	627	1	219	56	846	57
30–39	657	2	188	51	845	53
40–49	692	2	256	55	948	57
50–59	516	6	212	50	728	56
60–69	317		235	43	552	43
70–	131	1	127	24	258	25
All	3 310	21	1 387	425	4 697	446

Persons below 15 years of age were excluded Average participation was 84% (Table I) The majority of non participants were persons who had moved away from the area but were still in the registers persons who were away from home for a long period and persons who were hospitalized

The Lapps participated in a biologic and epidemiologic population study All the Lapps living in Inari commune were invited there were no age limits This report concerns only those Lapps who met the criterion that at least two but usually three generations of ancestors were Lapps On genetic and ethnic grounds the Lapps can be divided into four subgroups Fisher Lapps Mountain Lapps Nellim Skolts and Sevettı Skolts About 95% of the resident Skolt Lapps and about 50% of the resident most aboriginal (native genuine) Fisher and Mountain Lapps were investigated However a sufficiently large serum sample for the cholesterol and TG measurements was available only from 50% of these Lapps Since there were no statistically significant differences between the serum cholesterol and TG concentrations of the Lapp subgroups they were combined As a consequence of the sampling method many of the Lapps are closely related a there are also many complete families

Additional data Data describing the areas studied time of the study number of persons participation rates and length of fast are given in Tables I and II

METHODS

Blood samples The Finns were instructed to fast at least 4 hours and the Lapps to fast overnight before the examination All were asked about the actual length of the fast before the examination 29% of the Finns and 94% of the Lapps reported that they had fasted more than 12 hours (Table II) TG data of those who had fasted more and of those who had fasted less than 12 hours will be treated separately Blood samples from the Lapps were drawn directly after their arrival at the examination site

Since an oral glucose tolerance test was included in the multiphasic screening examination all Finns except known diabetics were given an oral glucose load graded according to BSA (60 75 or 90 g) directly after arrival The blood sample for cholesterol and TG determinations was drawn 1 hour later

Sera were separated and frozen immediately and stored at −20°C Sera from the Finns were analysed after 1–3 weeks of storage and sera from the Lapps after 1–2 years of

Table I *Population groups*

Commune	Geographic area	Type of population	Time of study	Persons studied Males	Females	All	Participation (%)
Inari	North Finland	Lapps	July–Aug 1969 and July–Aug 1970	382	446	828	(See the text)
Jaala	South Finland	Rural Finns	Aug –Sept 1970	859	893	1 752	78 5
Kuusankoski	South Finland	Semiurban Finns	Sept –Nov 1970	1 444	1 184	2 628	77 7
Kuusankoski (employees of Kymin Oy)	South Finland	Finns employed in industry	Nov –Dec 1970	1 308	447	1 755	93 0
Kajaani (employees of Kajaani Oy)	East Finland	Finns employed in industry	Feb –March 1971	1 571	353	1 924	94 5
Ristijarvi	East Finland	Rural Finns	March–April 1971	975	940	1 915	79 7
Suomussalmi (Ammansaari area)	East Finland	Semiurban Finns	May–June 1971	772	880	1 652	86 2
		All Finns (*Lapps excluded*)	Aug 1970– *June 1971*	6 929	4 697	11 626	83 9

Fig 1 Location of the population groups

semiurban Finns and the third Finns employed in industry The rural groups were mainly farmers and lumberjacks and their families Persons in the semiurban groups were mainly employed in manufacture trade transport and services or they were family members of such persons The industrial groups were plant and office workers in the paper and pulp industry

Our seventh population group consisted of Lapps living in Inari commune in North Finland (Fig 1 and Table I) The Lapps earn their living mainly from reindeer breeding fishing and agriculture (25) They form an ethnic group which differs from the Finns both genetically (13) and in mode of living

Selection of subjects We have attempted to study unselected samples of the general population Therefore we have not eliminated obese people people with CHD or diabetes people with an acute illness people whose test data have been outside the normal range or other groups The only groups *not included were persons who were hospitalized at* the time of the study and other non participants

All the six Finnish population groups participated in a multiphasic screening study which was carried out by the mobile clinic of the Social Insurance Institution In the rural and semiurban population groups all the persons or a representative sample of persons living within a specified area were invited by personal letter For the remaining two groups we invited all the employees of a paper and pulp factory Invitations were based on official population registers and on lists of employees respectively

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Serum Cholesterol and Triglyceride Concentrations of Finns and Finnish Lapps

I Basic Data

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Abstract Serum cholesterol and triglyceride (TG) concentrations have been studied in six Finnish population groups in South and East Finland and in one group of Finnish Lapps in North Finland Altogether 11 626 Finns aged 15 or above and 828 Lapps of all ages were studied Serum cholesterol and TG concentrations of the Lapp children were higher in the age group 0–4 than in the age groups 5–9 and 10–14 Mean serum cholesterol of girls aged 0–9 was higher than that of the boys The sex and age dependence of mean lipid values in the adults were in several respects similar to those observed in other industrialized populations The serum cholesterol mean rose with age to reach a maximum in age group 50–59 in men in age group 60–69 in women Among the adults women had lower cholesterol means than men only in age groups 30–39 and 40–49 The serum TG distribution was markedly skewed and logarithmic transformation brought it closer to normal Maximal log(TG) values (>12 hours fast) were attained by men aged 30–39 and by women aged 60–69 Men had considerably higher TG levels than women in middle aged groups Of all the sex and age groups studied the men aged 30–39 had the most skewed cholesterol frequency distribution Up to this age variability in men s cholesterol values also increased as reflected by an increasing S D As to women the cholesterol S D increased until the age of 60–69 In all the age groups above 14 years the variability of TG values was larger in men than in women but it was not age dependent

Mortality in coronary heart disease (CHD) among middle aged Finnish men is higher than among men of any other nationality The corresponding figures for Finnish women are also high, but not so extreme (47)

Prospective studies mainly on middle aged men have provided ample evidence of a statistical association between initial total serum cholesterol concentration and subsequent risk of CHD (26 27) The serum triglyceride (TG) concentration may predict CHD independently of the cholesterol concentration to judge from the few existing prospective studies (5 7)

The information available on the serum lipid levels of the Finns is sparse (1 27 43) In the study CHD in seven countries two cohorts of middle aged rural Finnish men were found to have higher cholesterol medians than any of the 14 cohorts in other countries (28)

We have attempted to obtain further information by measuring the serum cholesterol and TG concentrations of seven population groups in three regions of Finland One of these groups was made up of Finnish Lapps an ethnic group which differs from the rest of the Finns both genetically and in way of life The only children studied were Lapps

In the present report serum lipid concentration means standard deviations and the skewness of frequency distributions will be described and their relation to sex and age will be analysed Further analysis and data will be found in an accompanying report (4) and elsewhere (2)

SUBJECTS

The population groups Six Finnish population groups living in two geographic areas were studied (Fig 1 and Table I) In each area one population group represented rural Finns the second

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The estimated prevalence of hyperlipoproteinemia in the total series of males ($n=676$) aged 40–67 years was 25 8% with the following distribution among the three major types of hyperlipoproteinemia type II A 13% type II B 2% type IV 11% The prevalence of hyperlipoproteinemia has been recorded earlier in a randomized sample of males ($n=76$) aged 55 using the same technique (and the same technicians in this laboratory) The present data on males aged 40–67 years are in good agreement with those on males aged 55 type II A 12% type II B 1% types IV+V 11% A prospective study of males in O lo Norway gave a similar total prevalence of hyperlipoproteinemia 25% (E Gjone personal communication)

No attempt was made to determine whether hyperlipoproteinemia was primary secondary or acquired Earlier experience suggests that approximately 10% of the biochemically detected cases of hyperlipoproteinemia are of hereditary origin primary) An additional 2–5% might be secondary to somatic disorders (preferentially diabetes melli s or drug treatment In two males of the present series ($n=228$) diabetes mellitus was present the possible cause of secondary hyperlipoproteinemia type IV

The total estimated prevalence of hyperlipoproteinemia type IV was particularly high 14% in males aged 50 years (48–52 years) compared to 9 and 7% respectively in the 45 and 55 age groups Furthermore type IV appeared to be related to elevated BP In males with type IV the mean systolic BP was higher ($p<0.05$) than in the controls (Table II) The relationship between hypertension and hyperlipoproteinemia type IV has been further established in a study in progress including 6 hypertensive males (12) Moderate as well as re overweight appear to be common in hyperlipoproteinemia type IV (Fig 5) In males with type aged 50 mean weight was higher ($p<0.05$) n 50-year-old males in the normolipidemic series (Fig 4) In more selected series triglyceridemia has been linked to overweight to gain in body weight (2) and most recently to adipose tissue fat cell size (4)

These connections between hyperlipoproteinemia type IV elevated BP and overweight might suggest a causal relationship No statistical correlations were however found between serum lipids and BP (systolic or diastolic) in type IV Neither were weight serum lipids or BP significantly correlated in type IV

The current interest in hyperlipoproteinemias is primarily due to their relationship to the appearance of early CHD At the initial investigation in the present series ($n=228$) hyperlipoproteinemia was a common finding (Table I) among males with CHD ($n=13$) as well as in those with any abnormality on ECG ($n=42$) Type II B was particularly common among men with CHD ($p<0.001$) and type II A among men with pathologic ECG ($p<0.01$) In an earlier series (in which hyperlipoproteinemia was typed in the same way as in the present study) of males (aged <55 years) who had sustained an MI (11) type II B was ten times more common ($p<0.025$) and type IV twice as common ($p<0.05$) as in a control series

Primary prevention of CHD implies the detection and treatment of apparent risk factors in subjects with no signs of CHD at the time of the investigation Among the recognized risk factors elevated serum lipids the hyperlipoproteinemias are the least controversial Our present and earlier experience places particular emphasis on hyperlipoproteinemia type II B the combined elevation in serum cholesterol and triglycerides Various screening methods have been developed for the detection of hyperlipoproteinemia The one applied in the present series the Immunocrit test gave 58% false positives and 14% false negatives (Fig 1) indicating that more reliable tests are required Until it becomes possible to obtain complete serum lipid and lipoprotein analyses in large population samples one can focus on certain groups with high incidences of hyperlipoproteinemia The present study suggests that males free from CHD revealing one or more of the following attributes age 50 years elevated BP overweight abnormal ECG and/or diabetes mellitus should be the subject of further attention as to serum lipid and lipoprotein values Furthermore males who already show symptoms and signs of CHD should likewise for the sake of secondary prevention have their serum lipids and lipoproteins analysed

ACKNOWLEDGEMENTS

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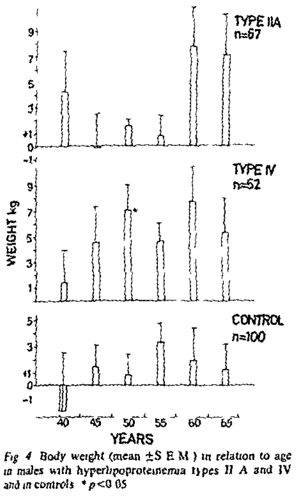


Fig 4 Body weight (mean ±S E M) in relation to age in males with hyperlipoproteinemia types II A and IV and in controls * $p<0.05$

($p<0.05$) in type II B ($p<0.001$) and in type IV ($p<0.05$) than in the controls Pathologic ECG was also more frequent in all hyperlipoproteinemias ($p<0.01$) in type II A ($p<0.01$) and in type IV ($p<0.05$) than in the controls

Blood pressure (Table II Fig 3) Mean systolic BP was highest in type IV and in CHD In type IV systolic BP was higher ($p<0.05$) than in the controls

Mean systolic and diastolic BP increased with age (Fig 3) in agreement with other studies (17) The means were highest for males with hyperlipoproteinemia type IV in age groups 60 and 65 years

Body weight (Table II Figs 4 and 5) Mean weight (expressed in relation to height see Methods) was highest in CHD and in hyperlipoproteinemia type IV In males aged 50 with type IV weight was higher ($p<0.05$) than in controls of the same age (Fig 4) In type IV weight showed a skew distribution (Fig 5) with a high incidence of males (>7% of all males with type IV) with extreme overweight (>+18 kg) and only a few lean subjects (weight <3 kg below 'ideal weight')

Correlations between serum lipids blood pressure and body weight In type IV no significant correlations were obtained between weight (expressed in relation to height) and serum cholesterol serum triglycerides or systolic or diastolic BP Neither were there any significant correlations between BP recordings and serum lipid values in hyperlipoproteinemia type IV

DISCUSSION

The aim of the present project was primary prevention of CHD through the detection and subsequent treatment of risk factors in 676 male employees attending an industrial preventive health center Among the risk factors serum lipid elevations were screened and subsequently biochemically typed for hyperlipoproteinemia Although it seems that the risk of CHD increases successively with increasing serum lipid values it was necessary—for the biochemical typing of hyperlipoproteinemia—to choose cutting points between normal and pathological values The cutting point for VLDL cholesterol was 35 mg/100 ml (corr sponding to a serum triglyceride value of proximately 180 mg/100 ml) and for LDL cholesterol 200 and 210 mg/100 ml below and above the age of 50 respectively (9) (corresponding to 280–300 mg/100 ml of total serum cholesterol)

Fig 5 Percentage distribution of body overweight (kg mean ±S E M) (for estimation see Methods) in relation to total number of males with hyperlipoproteinemia types II A and IV and controls

Table I *CHD and patholog c ECG in hyper l poproteinem a and in normol p demic matched controls*

	Type of hyperlipoprote nem a				
	II A (n 67)	II B (n 9)	IV (n 52)	Total (n 1 8)	Controls (n=100)
CHD					
%	4.5	33 3	9 6	8 6	2 0
n	3	3	5	11	2
p	n s	<0 001	<0 05	<0 05	
Pathol ECG					
%	?9 8	?2 ?	23 0	6 6	8 0
n	0	2	12	34	8
p	<0 01	n s	<0 05	<0 01	

BP was measured by the manometric method in the *right arm after 2 min rest with the subject in the sup ne* posit on The average of two observations was usually recorded Body we ght was measured in the naked state The nitially recorded we ght has been used and related to the figure obtained when height was subtracted by 100

Stat s ical methods Conventional statist cal methods were used for the calculation of means S D and S E M and for linear regression Student s *t* test was used to dy differences between the means of two groups al tat ve data were compared by means of χ^2 analyses r s exact probab lity test was used when necessary alue of $p \leq 0.05$ was cons dered to be statist cally ant

COMMENTS TO METHODS

r ng w h Immunocrit The Immunocrit method for s eening of serum lip d/l poprote n elevations is on the fact that serum containing monospecif c bod es to the β-lipoprote n prote n mo ety apo-poprote n B quant tatively precip tates pre β poprote ns (VLDL) together w th β-lipoprote ns (LDL) ut not chylom crons and α lipoprote ns (h gh density poprote ns HDL) The prec p tate s eas ly quant f ed by m asuring the he ght of the prec p tated lipoprote ns n a ap llary tube after packing by low speed centrifugat on he Immunocrit method was tested earlier and found to la e w th the sum of pre β and β lipoprote ns (19) In esent study a l m t of 3 3 mm prec p tate was chosen e entiate between normal and poss bly patho- l It became apparent from the subsequent serum nd l poprote n analyses that by exclud ng sub w th Immunocrit read ngs <3 3 mm a few cases w th hyperlipoprote nem a would be lost In a randomized samp e (n 87) of 38 w th Immunocrit <3 3 mm the s b equent serum l p d/l poprote n analyses revealed 1? t onal males (14%) w th hyperl poprote nemia Taking his te as representat e for the whole group (n=382) w h normal screen ng tests one could count on 40 5 add tional (F g 1) *males w th expected* hyper l poprote nemia

RESULTS

Prevalence of hyperl poprote nem a (F g 1) In ?80 males w th pathological screen ng tests (Im munocrit >3 3 *mm) the subsequent* serum l p d/ l poprotein analyses d sclosed biochem cal hyper l poprote nemia in 118 The d stribution among *the three major types of* hyperl poproteinemia was type II A 9 4% type II B 1 2% and type IV 7 0% The total number of cases w th found (n=130) and expected (n 40 5) hyperlipo proteinemia (see Comments to Methods) was 170.5 i e ?5 8% of the total series *In th s est* mat on type II A would account for 12 8% type II B for 1 9% and type IV for 11 1% of the cases

The age group 50 years (48–52 years) compris ing ?4% of the total series of males conta ned—although comparable n size with age groups 45 years and 55 years—the largest number of males (*n* ?1) *with type IV* (F g ?) The *found* and found+expected prevalence of type IV in this age group was 10 and 14% respectively compared w th 5 and 9% *in* age group 45 years and 3 and 7% in age group 55 years

Prevalence of CHD and patholog c ECG (Table I) Among a total of 228 males CHD was found in 13 (5 7%)—11 hyperl poproteinemias (*n* 128) and 2 controls (*n* 100) The data ind cate that CHD was more common in all hyperlipoprote nemias

Table II *Age systol c and d astol c BP and e ght in hyperlipoprotein e i a CHD and nor ol p d e i c matched controls*

	Type of hyperl poprotein em a				
	II A (n=67)	II B (n 9)	IV (n 5?)	CHD (n 13)	Con rols (n=100)
Mean age (y)	51 1	56 0	52 6	55 2	5? 3
Systolic BP (mmHg)					
Mean	141 ?	140 6	150 8	150 8	141 0
+S E M	? 3	7 8	4 6	6.5	? 3
p	n s	n s	<0 05	n s	
D astol c BP (mmHg)					
Mean	86 3	83 9	91 1	86 5	86 7
±S E M	1 1	3 4	3 0	3 4	1 ?
p	n s	n s	n s	n s	
We ght (kg)*					
Mean	+? 5	−0 4	+5 8	+7 ?	? 3
+S E M	1 1	? 3	1 1	1 7	? 9
p	n s	n s	n s	n s	

* For estimation see Methods

tein analysis performed on another blood sample (now obtained in the fasting state)

Series of males with hyperlipoproteinemia In the first round 118 men with hyperlipoproteinemia were confirmed by serum lipid/lipoprotein analyses (Fig 1) Twelve other men with hyperlipoproteinemia were included later (cf Comments to Methods) This report concerns the occurrence of hyperlipoproteinemia presence of CHD ECG changes elevated BP and body weight in a total of 128 men with hyperlipoproteinemia

Control series 100 normolipidemic matched control males were obtained as follows from the group of 237 men (Fig 1) with normal serum lipid/lipoprotein analyses The next man in the register after one with hyperlipoproteinemia belonging to the same age group and working in the same industry was selected as the matched control The series (total series hyperlipoproteinemic series and control series) were divided into the following age groups <43 43–47 48–52 53–57 58–62 and >62 years and these age groups were designated as 40 45 50 55 60 and 65 years In the normolipidemic matched controls the distribution among the age groups was similar to that of the total series of males (Fig 2)

METHODS

The screening method used for the detection of elevated serum lipid/lipoproteins was a semiquantitative immuno precipitation reaction Immunocrit (Hyland Laboratories Los Angeles USA) β L test (19) An upper limit of 3 3 mm was used as the borderline between normal and possibly pathological The Immunocrit was applied on non fasting samples as suggested (19)

Blood samples for subsequent serum lipid analyses were drawn in the morning in the fasting state Serum cholesterol was determined according to the Liebermann Burchard reaction by the method of Cramer and Isaksson (8) and serum triglycerides according to Carlson (6) Serum lipoprotein electrophoresis was performed on filter paper mainly according to the method described by Lees and Hatch (15) and on agarose gel principally according to the method described by Rapp and Kahlke (18) No attempts were made to quantify the lipoprotein bands

The serum lipoprotein patterns were typed biochemically according to the terminology of Fredrickson et al (9) with the inclusion of subtypes II A and II B as recommended (3) The patterns were judged biochemically from the visual evaluation of the lipoprotein electrophoresis in relation to the concomitant serum lipid analyses (cholesterol and triglycerides) A pre β lipoprotein elevation was considered when a distinct pre β lipoprotein band was present simultaneously with serum triglycerides ≥180 mg/100 ml The cholesterol content of β lipoproteins was estimated from the total serum cholesterol by subtracting the α lipoprotein cholesterol and the pre β lipoprotein cholesterol The latter was obtained from the serum triglyceride value multiplied by a factor of 0 20 for serum triglycerides <180 mg/100 ml and by a factor of 0 25 for serum triglycerides >180 mg/100 ml (10) In this equation α lipoprotein cholesterol was estimated to 50 mg/100 ml (10) when the lipoprotein electrophoresis showed a fairly normal amount of lipid stain at the place for the α lipoprotein In borderline cases the α lipoprotein cholesterol content was determined in serum after the complete precipitation of low density lipoproteins (LDL) and very low density lipoproteins (VLDL) (5) As normal upper limit 200 mg/100 ml was used for β lipoprotein cholesterol for men below the age of 50 and 210 mg/100 ml for men above that age (9) Repeated analyses were performed when serum lipid analyses and lipoprotein electrophoresis pattern did not agree No attempt was made to identify specifically the rare type III (9)

Clinical aspect The subgroup of males with CHD included those with symptoms of angina pectoris (AP) (n–9) and those who had sustained a myocardial infarction (MI) (n=4) AP was diagnosed from typical chest pains and ECG changes at rest In two cases a confirmative ECG at work was performed The MI diagnoses were obtained from hospital records

ECG at rest were read by one of us (D H) and were interpreted according to the Minnesota code Abnormal ECG included all ECG not registered as 1 0 0 In this presentation no attempt was made to differentiate ECG changes

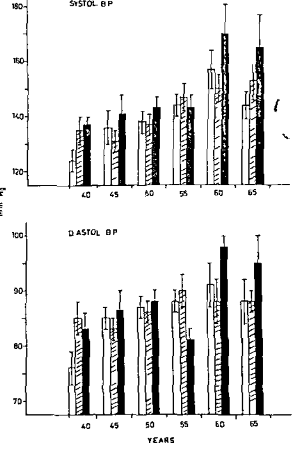
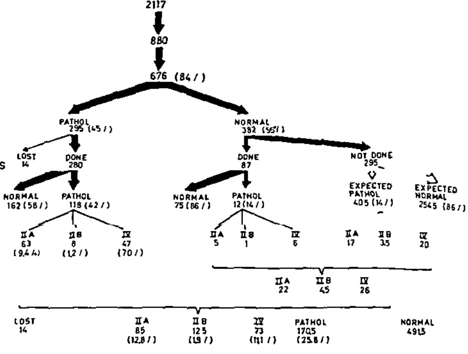
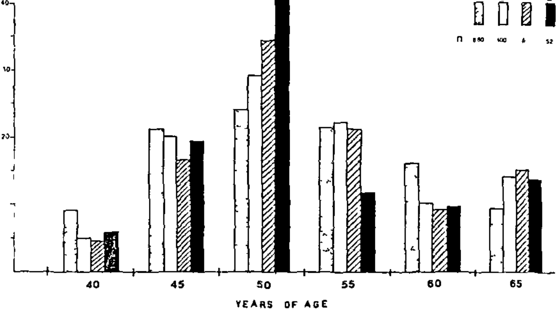


Fig 3 Systolic and diastolic BP (mean ±S E M) in relation to age The bars (from left to right) represent controls (n=100) males with hyperlipoproteinemia type II A (n–67) and type IV (n–52)



1 Outline of the study showing number of males ed as pathological and normal by screening and by /lipoprotein analysis Total prevalence of types of hyperlipoproteinemia estimated as found and ex pected is given at the bottom For further explanation see Comments to Methods



2 Distribution of hyperlipoproteinemia (% of total ber of males in each series) among different age ps 40 (<43) 45 (43–47) 50 (48–52) 55 (53–57) 60 (58–62) and 65 years (>62) Only found (verified) cases of hyperlipoproteinemia are included

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Prospective Study among Male Employees in Industry

I Prevalence of Hyperlipoproteinemia

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Abstract In a primary prevention study performed in an industrial preventive health center in Goteborg Sweden 676 male employees aged 40–67 years have been screened for the presence of elevated serum lipid/lipoprotein values and the estimated prevalence of hyperlipoproteinemia (according to the cut points) was 25 8% The hyperlipoproteinemias were distributed among the three major types II A II B and IV as 12 8 1 9 and 11 1% respectively The incidence of coronary heart disease (CHD) ($n=13$) pathologic ECG at rest ($n=42$) elevated systolic or diastolic BP and overweight were compared between 128 males with hyperlipoproteinemia and 100 matched normolipidemic controls from the same population Hyperlipoproteinemia type IV was most common in males aged 50 (48–52) years In type IV the mean systolic BP was higher ($p<0.05$) than in the controls and overweight was more frequent particularly in males aged 50 ($p<0.05$) There were however no statistically significant correlations among the variables serum lipids (cholesterol and triglycerides) BP (systolic or diastolic) and body weight (expressed in relation to height) in type IV CHD ($p<0.05$) and a pathologic ECG ($p<0.01$) were more common among males with hyperlipoproteinemia than in the controls The data from the present and earlier studies suggest that hyperlipoproteinemia should be looked for in particular among males aged 50 among males with elevated systolic BP and/or overweight, as well as among males already presenting signs or symptoms of CHD or a pathologic ECG

Coronary heart disease (CHD) is of multifactorial origin—more than 30 risk factors have been suggested Four of these have received a great deal of attention hyperlipidemia hypertension smoking and obesity

Among the hyperlipidemias hypercholesterolemia was early associated with CHD (16) and this relationship has been further established by more recent studies (14 20) Since 1960 serum triglycerides have been included in the list of CHD risk factors (1 7)

The water insoluble lipids i e cholesterol triglycerides and phospholipids are transported as miscellar or macromolecular lipoproteins in serum Elevated serum lipids hyperlipidemias are nowadays expressed more often as disturbances in the serum lipoprotein pattern (3 9)

With the aim of treating CHD risk factors a ventive health program for male employee group of small industries was started in Gote in 1970 The present report deals with the prevalence of biochemically typed hyperlipoproteinemias and their occurrence in relation to age CHD ECG changes BP and weight in 676 men aged 40–67 years

Request for reprints to A Gustafson MD Department of Medicine I Sahlgren s Hospital S-413 45 Göteborg Sweden

MATERIAL

The Gamlestaden Industrial Preventive Health Center in Goteborg Sweden serves 11 medium and small sized industries representing the light metal and food industries The present prospective study was performed over a year from Jan through Nov in 1971 The presence of CHD ECG changes elevated BP and overweight were recorded Males with risk factors were subsequently given advice for their correction and if necessary prescribed drug treatment

At the time when the study started the 11 industries had 2 117 registered employees 880 of whom were males aged 40–67 years (Fig 1) Eighty four per cent 676 males responded to the first call The age distribution of the whole series of 880 men is shown in Fig 2 These males were given a screening test (Immunocrit cf Methods) for the presence of elevated serum lipids/lipoproteins as well as a complete physical examination and ECG at rest Males with elevated serum lipids/lipoproteins as judged by the screening method were offered a serum lipid/lipopro-

Aldrich patients in the USA and in Europe Programs are also run for lepromatous leprosy patients by the WHO and CDC in the USA Similar studies are under way for chronic active hepatitis osteogenic sarcoma melanoma malignant measles congenital rubella and MS as well

A positive aspect of TF is the striking lack of side-effects Occasionally local reactions probably due to the chemotactic activity of TF and fever are seen There are also reports of more serious phenomena such as tumours sepsis and hemolytic anemia but they have appeared in patients with diseases like Wiskott Aldrich syndrome where they are common anyway Critical studies are obviously necessary for evaluation also of this side of TF immunotherapy

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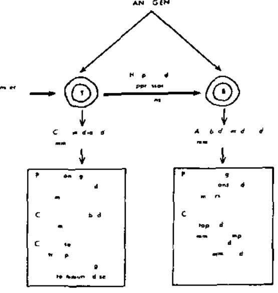


Fig 1

tivity TF also induces production of effector molecules lymphokines from the T lymphocytes of the recipient (11) Among these are lymphotoxin macrophage immobilizing factor (MIF) chemotactic factor (11) and interferon (5) These various phenomena can be used to detect TF activity but methods are needed for standardization of TF TF itself is not immunogenic it does not transfer any antigen and does not induce antibody formation

The obvious need for better therapy in several diseases has resulted in many therapeutic trials with TF although so little is known about its basic structure and function Most of these reports are empiric and rather anecdotal and controlled studies are still scarce Firstly TF has quite logically been applied in cases with various immunodeficiency diseases including defects in T lymphocyte function Positive effects have been reported especially the lethal Wiskott Aldrich syndrome Mostly the ms with infections have diminished but improvement of eczema and increase in platelet counts have also been noted Lawrence reports ... ally registered effects in 13 of 16 patients (11) In my own experience with three cases no or insignificant effects were obtained with TF (unpublished) Some patients with Swiss type and other forms of combined immunodeficiency ataxia telangiectasia Nezelof syndrome and various unclassified forms of immunodeficiency have also been treated In some cases in vivo as well as in vitro effects have been noted (11 14)

Immunotherapy with TF has been tried in various infectious diseases caused by intracellular parasites since cell mediated immunity is known to be the most important defense mechanism against such microbes Data have been presented from trials with patients diseased with lepramatous leprosy *miliary tuberculosis disseminated mucocutaneous* candidiasis and coccidioidomycosis as well as malignant measles subacute sclerosing panencephalitis (SSPE) multiple sclerosis (MS) disseminated vaccinia chronic active hepatitis herpes and congenital rubella In the reports on SSPE and lepromatous leprosy the results have been negative or limited in many cases but in others favourable findings were more common In malignant measles and in some series of patients with chronic candidiasis rather striking effects have been reported (11 14) Because cell mediated immunity is supposedly important in the defense against tumours TF has been tried in various forms of malignancies such as breast cancer melanoma sarcoma and acute leukemia In some cases regression of melanomas and sarcomas has been noted but no clinical benefit has been seen in acute leukemia In these patients as in those with immunodeficiencies and infections effects of TF could more often be registered by conversion of skin reactivity or by detection of lymphokines such as MIF than by improvement of the patients (11 14)

The recent concept that T lymphocytes play an important role controlling antibody forming B lymphocytes has suggested that diseases where malfunction of antibodies may be part of the pathogenesis can also be influenced by TF Thus some cases of rheumatoid arthritis and autoimmune hemolytic anemia given TF have been investigated so far without striking effects The suggestion of a T cell deficiency in atopic allergy made us give TF to a child with an extremely severe eczema and asthma In time relation to the TF injections the child showed a suggestive improvement of clinical status as well as laboratory parameters (12) This case seems quite suggestive but it should be stressed that this report as most others lacks controls and that the time has come for double blind clinical trials The real significance of TF immunotherapy may thus be definitively settled Such studies are already under way on a multi-center basis for Wiskott

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EDITORIAL

Transfer Factor—a New Form for Immunotherapy

With a simplification the effector mechanisms of the immune response can be divided into antibody and cell mediated immunity A typical example of a cell mediated reaction is the delayed hypersensitivity reaction against tuberculin Landsteiner and Chase (8) showed as early as in 1940 that this type of immune reaction could only be transferred from one individual to another through leucocytes This was in contrast to antibody mediated reactions which could be transferred with serum In 1954 Lawrence (9) demonstrated that transfer of delayed hypersensitivity reactions could also be effected with leucocyte extract Such material was given the designation transfer factor (TF) For several years this observation was honoured with little interest but with the advance of immunology it was brought back into focus again and now there is a boom in TF research This is due to the wide implications which TF may have for basic immunologic cell functions as well as through possibilities for a new type of therapy against many diseases like some infections autoimmune diseases, allergies and tumours

The reason why TF is now receiving so much attention is that it can influence those lymphoid cells the thymus dependent T lymphocytes which are the effector cells of cell mediated immunity It has been amply demonstrated during recent years not only that cell mediated immunity is of primary importance for our defense against certain infections and probably tumours but also that the T lymphocytes have an even more central position Obviously they play a key role in the immune response by being responsible for both cell mediated immune reactions and the helper and control functions crucial for the antibody forming B lymphocytes The way in which TF can influence the T lymphocyte is still not clarified The overall pattern may in a very simplified manner be illustrated as in Fig 1

Dysfunction of T lymphocytes may obviously play an important role in disease states such as immunodeficiencies involving T cells infections by intracellular parasites and tumours (Fig 1) Can TF improve the T lymphocyte function and positively influence the course of such diseases? Can it also be employed in the immunological diseases allergies immune complex diseases and some autoimmune diseases caused by antibodies synthesized by B lymphocytes because of erroneous or deficient T lymphocyte control?

These fascinating possibilities have boosted research on TF resulting in numerous reports on structure mechanisms of function and therapeutic trials TF has long resisted attempts at determination of its composition It has even been ridiculed saying that TF is known to function but has no been demonstrated to exist This research recently smeared by the lack of judgement young ambitious American student who pressur by an intensely competitive system presented falsified data on the composition of TF (2 3 13) Reliable and critical researchers have shown however that TF is a soluble dialysable lyophilizable polypeptide polynucleotide of molecular weight below 10 000 probably below 4 000 It is resistant to certain RNases DNases and proteases It can be kept active for years at −20 C but is inactivated at 56°C for 30 min (6 7 10)

The mode of function for TF is an enigma of considerable interest How can such a small molecule transfer specific immune reactivity in the form of delayed skin hypersensitivity against various antigens from bacteria viruses fungi parasites tumours and skin transplants? It has been suggested by Lawrence that TF may be an informational molecule or a specific gene derepressor making uncommitted lymphocytes antigen responsive (11) This question has not been settled by recent work and a demonstrable non specific adjuvant effect of TF on lymphoid cells has been proposed as an alternative mechanism (1 4)

Besides transferring delayed skin hypersensi

Table III *Serum cholesterol and log(TG) means and S D by sex age length of fast and ethnic group*

	Cholesterol (mg/100 ml)			Log(TG) (mg/100 ml)				
	Mean		S D	Mean (antilog within parentheses)			S D	
				Fast≤12 h	Fast>12 h		Fast≤ 12 h	Fast>12
Age (y)	Finns	Lapps	Lapps (0–14) Finns (≥15)	Finns	Finns	Lapps	Finns	Lapps (0–14) Finns (≥15)
Males								
0– 4		218	45			1 908 (81)		0 193
5– 9		187	34			1 818 (66)		0 173
10–14		203	35			1 796 (63)		0 165
15–19	200	198	36	1 968 (93)	1 888 (77)	1 844 (70)	0 179	0 160
20–29	233	238	44	2 079 (120)	2 010 (102)	1 955 (90)	0 198	0 200
30–39	264	270	50	2 139 (138)	2 107 (128)	2 036 (109)	0 207	0 202
40–49	278	281	48	2 171 (148)	2 106 (128)	2 032 (108)	0 208	0 183
50–59	282	282	49	2 150 (141)	2 105 (127)	2 059 (115)	0 192	0 175
60–69	273	280	48	2 129 (135)	2 086 (122)	2 019 (105)	0 207	0 205
70–	257	276	48	2 113 (130)	2 064 (116)	1 985 (97)	0 188	0 194
Females								
0– 4		232	50			1 868 (74)		0 171
5– 9		202	30			1 793 (62)		0 156
10–14		198	27			1 830 (68)		0 173
15–19	213	221	36	1 925 (84)	1 909 (81)	1 886 (77)	0 149	0 150
20–29	237	233	43	1 983 (96)	1 941 (87)	1 921 (83)	0 187	0 189
30–39	248	271	42	1 968 (93)	1 956 (90)	1 952 (90)	0 161	0 182
40–49	270	268	48	2 029 (107)	1 985 (97)	1 967 (93)	0 157	0 170
50–59	292	300	52	2 109 (129)	2 073 (118)	2 018 (104)	0 177	0 163
60–69	295	296	56	2 160 (145)	2 127 (134)	2 066 (116)	0 179	0 191
70–	284	255	52	2 207 (161)	2 114 (130)	1 986 (97)	0 256	0 182

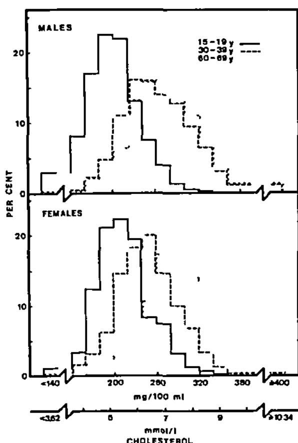
had several effects on the observations based on serum TG data (see Discussion and Table III) In particular the TG concentration was clearly lower after the longer fast

Means standard deviations and regression coefficients Means and S D by sex and age are given in Fig 2 and Table III Regression coefficients and constants of the regression equations for cholesterol and log(TG) on age in four age groups are listed in Table IV The sex and age trends apparent for children remained quite similar when 1 year age groups were examined Results for adult Finns and

Table IV *Regression coefficients (mg/100 ml/y) and constant terms (mg/100 ml) of the regression equations for cholesterol and log(TG) on age*

Cholesterol data for all persons studied and TG data for those who had fasted more than 12 hours

	No of persons		Cholesterol–age		Log(TG)–age	
Age (y)	All	Fasted >12 h	Regression coefficient	Constant term	Regression coefficient	Constant term
Males						
15–29	1 936	517	3 12	153 1	0 0163	1 609
30–44	2 317	585	2 25	185 1	− 0 0001	2 109
45–59	1 854	534	− 0 08	284 5	− 0 0023	2 224
60–	822	326	− 0 29	288 1	− 0 0015	2 180
Females						
15–29	1 366	369	1 65	192 7	0 0056	1 797
30–44	1 339	330	1 37	203 5	0 0078	1 860
45–59	1 182	326	2 37	163 4	0 0081	1 624
60–	810	363	− 0 33	315 6	− 0 0008	2 177



ʎ 3 Cholesterol frequency distributions for some sex and age groups

Lapps are rather similar (4) Notable trends observed to be associated with sex and age are listed below Only TG data for those who had fasted more than 12 hours are considered

The cholesterol mean of the girls aged 0–9 was 19 mg/100 ml (0 49 mmol/l) higher than that of the boys ($p<0\ 01$)

Among the adults women had lower cholesterol means than men only in the age groups 30–39 and 40–49 ($p<0\ 01$) In the age groups 15–19 50–59 60–69 and ≥70 women had higher cholesterol means than men ($p<0\ 01$)

Women in the age groups 20–29 30–39 40–49 and 50–59 had logarithmic TG means lower than men ($p<0\ 01$) In the age groups 30–39 and 40–49 the difference was marked Older women (60–69 ≥70) had higher means than men ($p<0\ 01$)

In all the age groups above 15 years women had a lower observed S D for log(TG) than men

Observed cholesterol and logarithmic TG means for both sexes had a minimum at 5–9 or 10–14 years

The cholesterol means of boys and girls aged 0–4 were significantly higher than the respective means in age group 5–9 (boys $p<0\ 05$ girls $p<0\ 025$) The cholesterol mean of age group 0–4 was significantly higher than the mean in age group 10–14 in girls ($p<0\ 01$) but not in boys ($p<0\ 20$) Although the log (TG) level was higher in age group 0–4 than in age groups 5–9 or 10–14 none of the differences between age groups reached statistical significance

After the age of 15–19 the cholesterol means of both men and women rose steeply and reached a maximum for men at 50–59 and for women at 60–69 The observed S D were fairly constant in men from age 30–39 onwards whereas in women they increased until the age of 60–69

After the age of 15–19 the logarithmic TG mean of men rose steeply and reached a maximum at 30–39 In the group with the shorter fast the maximum was reached later on In women a rapid rise of mean log (TG) began after the menopause and reached a maximum at 60–69 The length of the fast did not materially affect this age dependence The S D among adults were lowest in age group 15–19 but there was no clearcut age dependence in the older age groups

Skewness of frequency distributions The men aged 30–39 had the most skewed cholesterol dis

Table V *Skewness of serum cholesterol TG and log(TG) frequency distributions*

Cholesterol data for all Finns and TG data for those Finns who had fasted more than 12 hours

	Skewness		
Age (y)	Cholesterol	TG	Log(TG)
Males			
15–19	0 43**	1 40**	0 02
20–29	0 60**	1 50 *	0 15
30–39	1 05**	2 67**	0 41**
40–49	0 43**	3 47**	0 30
50–59	0 66**	1 32**	− 0 02
60–69	0 23*	3 80**	0 72**
70–	0 08	1 86 *	0 42 *
Females			
15–19	0 53**	1 42**	0 01
20–29	0 68**	2 54**	0 23
30–39	0 54**	1 77**	0 37*
40–49	0 81**	3 01**	0 49**
50–59	0 77**	1 14**	0 03
60–69	0 86**	1 67**	0 26
70–	0 96**	1 52**	0 01

* Almost significantly different from 0 ($p<0\ 05$) ** significantly different from 0 ($p<0\ 01$)

tribution (Fig 3 and Table V) The skewness of log(TG) was positive in most sex and age groups (Table V)

DISCUSSION

Factors complicating interpretations Many investigators have studied the serum lipid levels of population groups from which obese persons persons with some disease(s) or some other groups had been eliminated The variety of criteria used makes it difficult to compare the results with each other or with the present findings We have tried to study the serum lipid concentrations of unselected Finnish populations rather than those of any selected group

Another factor complicating interpopulation comparisons is the observed variability in laboratory accuracy (45) Our cholesterol values were some 5% higher and TG values equal to those of the Lipid Standardization Laboratory of the Center for Disease Control

Seasonal variations of a variable magnitude with summer values usually lower than winter values apparently occur in the serum cholesterol (8 42) and TG (8) concentrations of at least some population groups With regard to serum TG there is also evidence pointing in the opposite direction i e suggesting that winter values may be much lower than summer values (16) Previous studies on small Finnish groups have indicated that serum cholesterol medians may (30 41) or may not (31 41) be significantly lower in the summer than in the winter

We have examined our preliminary results (Aromaa et al unpublished) from all areas studied consecutively in these multiphasic screening examinations in the period 1969–72 The cholesterol means of 35 000 analyses and the log(TG) means of 21 000 analyses are presented in Fig 4 by month of study Age adjusted cholesterol means fell during the spring and were some 10–15 mg/100 ml lower in June than during most other months of the year Log(TG) means adjusted for age and length of fast did not follow any clearcut seasonal pattern In the present study lipid data for the Lapps were collected in July–Aug and data for the other Finns rather evenly during the year (Table I) Consequently the estimate of the cholesterol level of the Lapps may be too low

Skewness of frequency distributions Serum cholesterol (8 22 34) and TG (8 14) frequency distributions have been found to be positively skewed in several previous population studies The same was observed now with the Finns (Table V) In addition we found the log(TG) distribution to be positively skewed Variations with sex and age in the distributions will be discussed in the following

Variations with sex and age in children In our investigation a group of 205 children of various ages was included All of these children belonged to a rather special population group the Lapps Furthermore a lot of them were closely related Therefore the results may not be generally valid

Lapp girls in the age range 0–9 had higher cholesterol means than boys (Fig 2 and Table Similar differences between boys and girls have been observed previously (11 18 23) and they seem to be present already during the first months of life (15)

The cholesterol and log(TG) means of boys and girls in the age group 0–4 were higher than those of the older children (Fig 2 and Table III) Again with regard to serum cholesterol some similar observations can be cited (23 32 35) The cholesterol and log(TG) means started to rise again after the age of 5–9 or 10–14 (Fig 2 and Table III) In previous population studies a rise with age has been reported to start during the age span of 6–7 (21) 5–10 (35) 6–11 (18) or 15–19 (23) while some investigators report no change between 5 and 14 years (20) The pattern of rather high cholesterol and TG values in young children followed by lower ones with a rise starting somewhere between 5 and 15 years may be a general phenomenon

Variations with sex and age in adults Variations in serum cholesterol means and S D have been studied in several large industrialized populations (22 23 34 36) and the corresponding TG statistics in selected or smaller but otherwise similar populations (8 19 33 44 46) The sex and age trends observed in these previous studies are in many respects similar to those reported here and elsewhere for the Finns (1)

We observed maxima both when cholesterol means (Fig 2 and Table III) and when the skewness of cholesterol frequency distributions of men (Fig 3 and Table V) were examined as a function of age At 30–39 years skewness was maximal for men and at this age 13 3% of the men but only 5 3% of the women had a serum cholesterol concentration above 320 mg/100 ml (8 28 mmol/l) (Fig 3)

There were clear sex and age determined differences in serum TG means and S D (Fig 2 and T III) Men aged 30–39 and 40–49 had consi

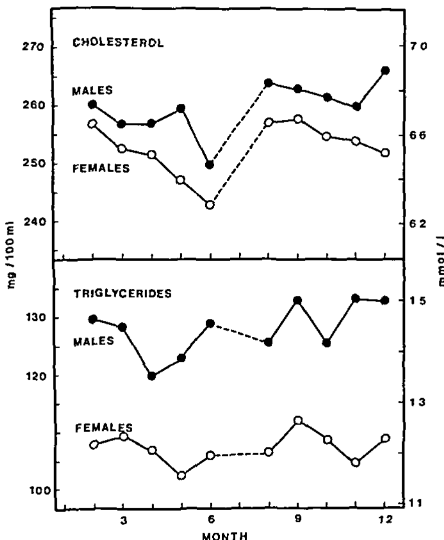


Fig 4 Mean serum cholesterol and mean serum log(TG) by month of study Triglycerides are presented as antilogarithms of log(TG) means Cholesterol means are age adjusted log(TG) means are adjusted for age and length of fast Data are based on 35 000 cholesterol and 21 000 TG analyses (Aromaa et al unpublished)

higher TG levels than women In all the age groups over 14 years the S D of log(TG) was larger in men than in women

Mean cholesterol levelled off or started to fall after the age of 50 in men and 60 in women Mean log(TG) behaved in much the same way although the changes with advancing age were less marked Both of these phenomena as well as a maximum skewness and S D of the cholesterol distribution of men at fairly early ages may be due to an increased mortality rate in persons with high cholesterol and/or TG levels

Triglyceride levels and length of fast It is of interest that the difference in serum TG concentrations between men and women was larger after the shorter fast (≤12 h) The difference between the values of young and old women was similarly affected (Fig 2 and Table III) This is in accordance with the more rapid TG clearance in women of fertile age (40) which may also explain at least part of the difference between TG levels in fasting men and women

The serum lipid levels of the Finnish population The cholesterol level of the Finnish men has been observed to be high in comparison with several other population groups (27) On the basis of the data presented above it is evident that both men and women in Finland have high cholesterol and TG levels A description of the prevalence of hyperlipidemias in Finland interpopulation comparisons and a discussion of the relevance of the findings with respect to differences in the incidence of CHD will be given in a subsequent paper (4)

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Serum Cholesterol and Triglyceride Concentrations of Finns and Finnish Lapps

II Interpopulation Comparisons and Occurrence of Hyperlipidemia

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Abstract Only small differences in serum cholesterol means and somewhat larger but still modest differences in log(TG) means were observed when seven Finnish population groups were compared Semiurban and industrial groups had the highest triglyceride levels Cholesterol levels tended to be lower in South Finland than in East or North Finland and lowest in semiurban population groups Between 15–59 years of age rural Finns of both sexes had higher and after this age lower cholesterol means than the Finns in the other population groups The highest cholesterol levels below the age of 60 were observed in a rural population group in East Finland The Finnish Lapps and the rural Finns had nearly identical serum lipid levels in spite of the differences in genetic background and way of life The serum lipid concentration means were quite similar to those reported from the other Nordic countries Denmark Norway and Sweden The differences between the populations of the Nordic countries are no larger than those observed between the Finnish population groups However no population in Scandinavia or elsewhere can confidently be said to have higher serum lipid levels than the Finns The similarity of the lipid levels observed in different areas in Finland and in the larger Nordic area is remarkable in view of the large differences in the incidence of coronary heart disease and in mortality rates It is conceivable that part of the variations in disease rates is attributable to differences in the prevalence of hyperlipidemias although the present study does not prove this hypothesis Such differences could occur in spite of similarities in the lipid concentration means Prevalences of hyperlipidemias in Finland are given

Mortality in coronary heart disease (CHD) in Finland is known to be particularly high For men as well as for women it is much higher than in the other Scandinavian countries Denmark Norway and Sweden (8) Among men aged 45–54 CHD mortality is 3 4 times higher in Finland than in Sweden (51) Large regional differences in total mortality (34) and CHD mortality (13) are also observed in Finland These differences are particularly large among men The incidence of CHD in middle aged rural men in East Finland is roughly twice as high as among men in the West (26) The differences in morbidity and mortality rates could be due to differences in the level or prevalence of the principal risk indicators of CHD

Few efforts have been made to compare the serum lipid levels of Finnish and other Scandinavian populations There are also only a few studies comparing serum lipid levels of various Finnish population groups Karvonen et al (24) found that middle aged men in rural East Finland had slightly higher cholesterol levels than men in the rural West Both these Finnish groups had higher serum cholesterol medians than any of the other cohorts of the seven countries study (30) The limited information available suggests that the prevalence of the common hyperlipidemia phenotypes (IIa IIb and IV of Beaumont et al (6)) may be high in Finland (1 33 37)

We have studied and compared the serum cholesterol and triglyceride (TG) concentrations in seven Finnish population groups and the results have also been compared with published data f other countries Three population groups studied in South Finland were male CHD

Table I *Age-adjusted serum cholesterol and log(TG) means (mg/100 ml) in seven populations*

Data are given for the populations uncombined and combined in two ways Cholesterol concentrations are given for all the persons studied and log(TG) concentrations for those who had fasted more than 12 hours (antilog within parentheses) *F* test values which indicate the significance of differences are given after each data group **significant differences ($p<0$ 01)

Criteria for grouping populations	Cholesterol No of subj	Cholesterol Mean	Log(TG) No of subj	Log(TG) Mean
Locality (uncombined populations)				
Men				
Inari (Lapps North)	280	262	258	2 001 (100)
Jaala (rural South)	859	257	340	2 012 (103)
Kuusankoski (semiurban South)	1 444	254	491	2 082 (121)
Kuusankoski (industrial South)	1 309	259	476	2 091 (123)
Kajaani (industrial East)	1 571	261	310	2 087 (122)
Ristijarvi (rural East)	975	266	271	2 030 (107)
Suomussalmi (semiurban East)	772	257	74	2 055 (114)
F test	*F* (6 7 162)=8 08**		*F* (6 2 173)=12 33**	
Women				
Inari (Lapps North)	343	264	328	1 976 (95)
Jaala (rural South)	893	261	377	1 971 (94)
Kuusankoski (semiurban South)	1 184	256	426	2 028 (107)
Kuusankoski (industrial South)	447	262	156	2 027 (106)
Kajaani (industrial East)	353	267	54	2 034 (108)
Ristijarvi (rural East)	940	272	289	2 025 (106)
Suomussalmi (semiurban East)	879	255	86	2 029 (107)
	F (6 4 993)=14 38**		*F* (6 1 670)=5 58**	
area				
Finland (Lapps)	280	262	258	2 001 (100)
East Finland	3 318	261	654	2 057 (114)
South Finland	3 611	256	1 307	2 065 (116)
F test	*F* (2 7 188)=10 8 *		*F* (2 2 198)=11 9**	
Women				
North Finland (Lapps)	343	264	328	1 976 (95)
East Finland	2 173	265	428	2 022 (105)
South Finland	2 524	259	959	2 009 (102)
F-test	*F* (2 5 019)=9 5**		*F* (2 1 694)=5 9**	
Type of population				
Men				
Lapps	280	262	258	2 001 (100)
Rural Finns	1 834	262	611	2 018 (104)
Semiurban Finns	2 216	255	564	2 077 (119)
Finns employed in industry	2 879	260	786	2 088 (123)
F-test	*F* (3 7 182)=9 2 *		*F* (3 2 192)=23 6**	
Women				
Lapps	343	264	328	1 976 (95)
Rural Finns	1 833	267	666	1 993 (98)
Semiurban Finns	2 064	255	511	2 034 (108)
Finns employed in industry	800	264	210	2 026 (106)
F-test	*F* (3 5 013)=19 2**		*F* (3 1 688)=6 6**	

Table II *Mean serum cholesterol and log(TG) (mg/100 ml) by sex age and type of pop lat on*

Cholesterol concentrat ons are g ven for all the persons stud ed and log(TG) concentrat ons for those who had fas ed more than 12 hours

	Age (y)	Rural populat on groups: Inari Lapps (North)	Jaala (South)	Rist jarvi (East)	Sem urban popu lat on groups: Kuusan koski (South)	Suomus salm (East)	Industrial popu lat on groups: Kuusan koski (South)	Kajaan (E t)
Cholesterol								
Men	15–19	198	207	212	194	190	211	1
	20–29	238	234	240	225	234	33	34
	30–39	269	256	279	261	262	263	63
	40–49	281	277	282	274	278	276	282
	50–59	282	282	294	270	281	81	286
	60–69	280	267	271	275	275	84	73
	70–	276	253	256	260	295		
Women	15–19	221	226	234	204	203	213	250
	20–29	233	239	247	231	237	233	240
	30–39	271	248	263	242	243	45	254
	40–49	268	272	286	259	263	271	276
	50–59	300	89	299	286	286	299	95
	60–69	296	290	297	295	286	303	31
	70–	254	275	284	293	285		
Log(TG)								
Men	15–19	1 844	1 873	1 824	1 978	1 852	1 991	1 948
	20–29	1 955	1 932	1 984	2 0 0	1 940	2 063	006
	30–39	2 036	2 004	2 054	2 126	2 198	2 121	109
	40–49	2 032	2 048	2 076	2 152	2 043	2 086	14
	50–59	2 059	2 052	2 086	2 089	2 180	2 176	2 154
	60–69	2 019	2 066	2 033	2 105	2 008	2 165	2 083
	70–	1 985	2 030	2 128	2 059			
Women	15–19	1 889	1 889	1 880	1 868	1 877	1 891	
	20–29	1 921	1 882	1 944	1 961	1 954	1 945	1 989
	30–39	1 952	1 971	1 971	1 97	1 946	1 947	2 000
	40–49	1 967	1 939	2 015	1 994	2 003	2 007	1 962
	50–59	2 018	2 027	2 057	2 112	2 103	2 091	2 090
	60–69	2 066	2 070	2 177	2 124	2 300	2 196	2 166
	70–	1 986	2 059	2 161	2 149			

is about average and three groups in East Finland where CHD mortality is higher than average (13) The seventh populat on was made up of F nn sh Lapps in North F nland The Lapps are an ethn c group differing from the rest of the Finns both genet ically and in way of l fe (17)

Basic informat on on the serum cholesterol and TG levels of the Finns and the Finn sh Lapps has been presented in a preced ng report (2) and further data will be found elsewhere (3)

SUBJECTS AND METHODS

Seven populat on groups l v ng n F nland were stud ed The g oups have n several cases been comb ned nto larger groups accord ng to the geograph c area or the type of population to which they belong (Table I) In the present report cholesterol data for all the persons stud ed and TG data for those who had fasted more than 12 hours w ll be analysed A detailed account of the populat on groups and most methods has been given n a prev ous report (2)

Due to the marked skewness of the TG d stribut on a logarithmic transformat on was employed (2) In add t on to the logarithm c mean values the r ant logarithms wh ch are close to the med ans of the original d stribution will generally be g en

Regression analys s was used to compute the age adjusted cholesterol and log(TG) means for each popula tion group (46) Each 5 year age g oup and each populat on group were represen ed by a (0 1) explanatory variable in the regression analys s The s at s cal s gn f ance of the d fferences of the (age adjusted l p d levels of the popula t on groups and nteract ons between the age g oups and popula on groups were tested by means of F sher s *F*

In calculations dealing w th correlat on coeff transforma on (44) was employed The hc

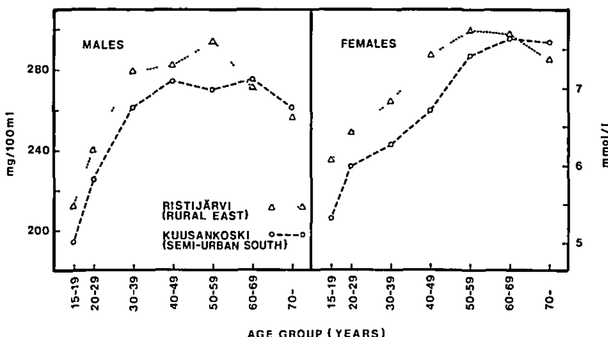


Fig 1 Mean serum cholesterol in the two population groups with the largest difference in serum cholesterol levels

tion parameters (correlation coefficients skewness) erent age groups was tested using an approximate test which is applicable for large samples (44) If the test did not indicate inhomogeneity the parameter estimates of different age groups were combined by weighing each with its error variance (44) When dealing with skewness this was also done despite any inhomogeneity

RESULTS

Interpopulation comparisons Age adjusted serum cholesterol and log(TG) means in the seven popula tion groups and in population groups combined by geographic area and by type of population are given in Table I Age group specific means in all the population groups are given in Table II The observed variations in age adjusted means between the subgroups were statistically significant in all the groupings of Table I both in men and in women In absolute terms however the differences in the cholesterol means were modest the largest differ ence between two male populations was 12 mg/100 ml (0 31 mmol/l) and between two female popula tions 17 mg/100 ml (0 44 mmol/l) The differences in age adjusted TG means were relatively larger The largest difference between the antilogarithms of log (TG) means of two male populations was 23 mg/100 ml (0 26 mmol/l) and of two female populations 14 mg/100 ml (0 16 mmol/l)

However age adjusted means conceal some im portant findings When the regression model was fitted to the cholesterol data, a statistically signifi cant interaction between age group and type of population was observed in men and in women Thus the age dependence of mean serum choles terol was different in various types of population and at 15–59 years of age rural Finns tended to have higher serum cholesterol means than semiurban and industrial Finns and lower means thereafter The largest age group specific differences are illustrated in Fig 1 in which the two population groups with the largest difference in cholesterol levels are compared

The variations in means between types of popula tion were larger than between geographic areas This was true for both men and women cholesterol and log(TG) age adjusted data and data for separate age groups After comparing results for different types of population the following observations were made (Figs 1 and 2 Tables I and II)

The lipid values of the Lapps and the rural Finns were very similar

Most age groups below 60 of the Lapps and the *rural Finns had higher cholesterol means th in cor* responding age groups of the other types of popula tion At higher ages the situation was reversed

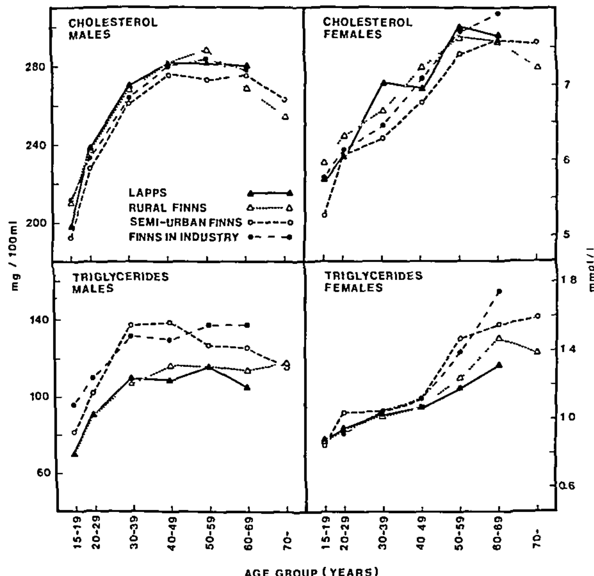


Fig 2 Mean serum cholesterol and mean serum log (TG) by type of population

The semiurban Finns had the lowest cholesterol means

The Lapps and the rural Finns had the lowest log (TG) means

Log(TG) means of women were very similar in all types of population up to the menopause There after mean values were lowest in the Lapps

With regard to different geographic areas the following observations were made Cholesterol levels were slightly lower in South Finland than in East or North Finland Age specific mean cholesterol values of men and women aged 15–19 20–29 30–39 and 40–49 tended to be higher in the East than in the South in every type of population Age adjusted mean log(TG) values were lowest in North Finland i e the Lapps and quite similar in South and East Finland This finding was confirmed for Lapp men in all the age groups

Finally there was a rather unexpected tendency for high cholesterol means and low log(TG) means to occur in the same population groups (Fig 2 and Table I)

The conclusions drawn above can be confirmed by examining the mean values in 10-year age groups in all the seven original population groups although a few inconsistencies exist (Table II)

Table III *Prevalence (%) of hyperlipidemias in Finns*

Calculations are based on results of all the Finns who had fasted more than 12 hours

Age (y)	Normal[a]	Type of hyperlipidemia IIa[b]	IIb[c]	IV[d]
Men				
15–19	94.2	2 1	0 0	3 2
20–29	74 1	7 9	6 1	11 9
30–39	48 7	22 0	16 3	13 0
40–49	47 6	25 5	17 2	9 7
50–59	41 7	30 2	16 8	11 2
60–69	49 3	30 0	15 3	9 4
70–	59 3	19 8	11 0	9 9
Women				
15–19	92 7	6 0	0 0	1 3
20–29	79 9	13 2	3 7	3 2
30–39	73 9	16 0	4 8	5 2
40–49	60.5	30 1	5 5	3 9
50–59	39 0	37 6	18 8	4.2
60–69	33 6	33 2	23 4	9 8
70–	38 3	33 6	21 1	7 0

[a] Cholesterol≤280 mg/100 ml (7.24 mmol/l) TG≤160 mg/100 ml (1 81 mmol/l)
[b] Cholesterol>280 mg/100 ml (7 24 mmol/l) TG≤160 100 ml (1 81 mmol/l)
olesterol>280 mg/100 ml (7 24 mmol/l) TG>160 100 ml (1 81 mmol/l)
Cholesterol≤280 mg/100 ml (7 24 mmol/l) TG>160 g/100 ml (1 81 mmol/l)

Occurrence of hyperlipidemia The prevalence of hyperlipidemias among the combined Finnish populations was calculated in the manner of Carlson and Böttiger (11) from the cholesterol and TG values (Table III) By these rather arbitrary and very low criteria phenotype IIa (pure hypercholesterolemia) was the most common type followed by type IIb (high cholesterol and high TG) In the age groups 20–29 30–39 and 40–49 types IIb and IV (pure hypertriglyceridemia) were more common among men than women This is due to the relatively high serum TG level of men in these age groups

The frequency with which phenotype IIb occurs is related to the positive correlation between cholesterol and log(TG) concentrations (Table IV) The correlation coefficients did not vary significantly with age in the groups consisting of Finnish men Finnish women Lapp men and Lapp women The correlation coefficients for Finnish men and for Lapp women were almost significantly higher ($p<0.05$) than for Finnish women These correlation coefficients were of the same order in all the Finnish population groups

Table IV *Cholesterol on log(TG) correlation coefficients and their 95% confidence intervals*

Coefficients (all age groups combined) are based on results in persons who fasted more than 12 hours

Population	Correlation coefficient	Confidence interval
Male Finns	0 409	0 390–0 478
Female Finns	0 333	0 294–0 398
Male Lapps	0 377	0 283–0 464
Female Lapps	0 475	0 397–0.546

The prevalence of hyperlipidemias was also examined separately in each population group In general the results were what might be expected considering the difference in cholesterol and TG levels Thus type IIa was most prevalent among male Lapps and among male and female rural Finns Types IIb and IV were most prevalent among semiurban Finns and Finns employed in industry

The occurrence of hyperlipidemias in Finns is further described by the cholesterol and TG fractiles given in Table V The fractiles also demonstrate variations with age and sex which are in many respects similar to those described previously with regard to mean values (2)

Table V *Cholesterol and triglyceride fractiles (mg/100 ml) by sex and age*

Cholesterol data for all the Finns and triglyceride data for all the Finns who had fasted more than 12 hours

	Age (y)	No of subj	Fractile 50%	95%	99%
Cholesterol					
Men	15–29	1 936	218	303	348
	30–44	2 317	264	356	408
	45–59	1 854	276	364	418
	60–	822	270	348	399
Women	15–29	1 366	223	303	348
	30–44	1 338	252	332	381
	45–59	1 182	283	373	428
	60–	809	289	381	438
TG					
Men	15–29	517	90	208	296
	30–44	585	126	289	390
	45–59	534	130	246	381
	60–	326	118	289	399
Women	15–29	369	81	158	296
	30–44	330	86	188	310
	45–59	326	114	208	283
	60–	363	131	283	428

DISCUSSION

Comparisons between different Finnish populations When a probable difference of 5 % in accuracy (2) is taken into account our cholesterol medians (Table V) are very similar to those reported earlier for middle aged Finnish men by Karvonen et al (24) Some other Finnish studies have yielded cholesterol means similar to ours (25 27 43) or slightly lower than ours (28 32 42)

Karvonen et al (24) also found that middle aged men in rural West Finland had cholesterol medians which in different age groups were 5–17 mg/100 ml (0 13–0 44 mmol/l) lower than those in men in rural East Finland Quite similar results can be extracted from our data except that we studied rural men in the South rather than in the West (Tables I and II)

The observed differences between the serum cholesterol levels of our population groups were small but statistically significant When these results are interpreted the possible effect of seasonal variations and long term fluctuations in laboratory accuracy (bias) – in spite of quality controls – must be taken into account (2) All the small differences in the age adjusted cholesterol means (Table I) may not be true differences

The age dependence of the cholesterol levels was found to be different in different population groups (Figs 1 and 2) Rural population groups had a higher level below the age of 60 and a lower level at older ages This must be taken as evidence of real interpopulation differences in cholesterol means in several sex and age groups It is also clear that these interpopulation differences must be modest and it appears that they are mainly differences between types of population (rural semiurban or industrial) (Table I) In fact the differences between the geographic areas (South and East) were almost negligible in the semiurban and industrial population groups but larger differences were observed between mean cholesterol levels of the two rural groups with higher values in the East (Table II)

The sizeable interpopulation differences in log (TG) means suggest that the rural Finnish and Lapp population groups of this study do indeed have lower serum TG levels than other types of population (Fig 2 and Table I) This could perhaps be related to the fact that people in the rural population groups are physically more active and men – but not women – are lean compared with persons belonging to semiurban and industrial population groups

The TG levels in women belonging to different types of population groups are relatively low and quite similar up to the menopause (Fig 2) The difference in age adjusted means between Lapp and Finnish women is mainly due to the low TG levels of Lapp women after the menopause Fairly low serum TG concentrations in premenopausal women could be due to the rapid clearance of serum TG in them (41)

In view of the differences in genetic background (17) and way of life (22) it is quite surprising tha h Lapps and the rural Finns have nearly iden i serum lipid levels (Fig 2 Tables I and II)

It may be concluded that the differences between the serum cholesterol levels of various population groups in Finland are small and that the differences between the serum TG levels are slightly larger Rural population groups in East Finland may have higher mean cholesterol concentrations than rural population groups in South and West Finland but this is true only below the age of 60 At older ages the situation is reversed Semiurban and industrial groups have the highest TG levels

Comparisons between Finnish and other Scandinavian populations Several studies reporting serum cholesterol means similar to those found in Finland (Table I) have been carried out in Sweden (12 48) Norway (7 35) and Denmark (16 21) (Table

Table VI *Age adjusted serum cholesterol and log(TG) means (mg/100 ml) in Finns and other Scandinavians*

For cholesterol age adjustment was made using the numbers of All Finns and for log(TG) using the numbers of Finns fast>12 hours in the age groups 20–69 years in Table II of Aromaa et al (2)

	Cholesterol		Log(TG) (antilog within parentheses)	
	Men	Women	Men	Women
Denmark	257	265	2 074 (106)	1 970 (83)
Finland[b]	265	266	2 084 (121)	2 018 (104)
Norway	244			
Sweden[d]	268	273	2 064 (116)	1 982 (96)

[a] Healthy non-obese persons who had fasted 12 hours (5)
[b] Cholesterol means are for all the Finns and log(TG) means for the Finns who had fasted more than 12 hours (present study)
[c] Healthy persons (35)
[d] Calculated for the entire population of Carlson and Lindstedt (12) Obese and sick (but not hospitalized) persons were included All had fasted overnight

VI) In some smaller studies somewhat lower results have been obtained (36 49)

With regard to Scandinavian TG levels the situation is similar Values close to the Finnish ones have been reported for men and women in Denmark (4) men in Sweden (12 38 49) and women in Sweden (49) (Table VI) Other studies have yielded somewhat lower values for men (14 16 20 21 35) or women (12 14 16 21 35)

It is quite remarkable that the serum lipid values in various Finnish and other Scandinavian populations are so similar The similarity extends to such special groups as the Finnish Lapps of this study and Greenlandic Eskimos living in Denmark (while Eskimos in Greenland have lower values) (4) Differences between various Nordic populations (Table VI) are only slightly larger than those observed within Finland (Table I)

Even after allowance for possible differences in accuracy (bias) between different laboratories cholesterol values reported from industrialized countries outside Scandinavia (18 19 23 30 38 39) seem to be lower than Finnish or other Scandinavian es both for men and for women

, of TG values are hampered by the data from large populations and by the many ways in which data have been presented There are studies from industrialized countries that report TG levels similar to ours or even higher (5 10 18 38 45) and others that suggest clearly lower levels (19 50)

It appears that so far no population has been described which could confidently be said to have higher serum cholesterol or TG concentrations than the Finns However in industrialized countries—especially in Scandinavia—there are populations with closely similar lipid levels

Prevalence of hyperlipidemias The prevalences of hyperlipidemias are difficult to describe since there are no generally accepted rigorous definitions of the various types Cholesterol and TG fractiles (Table V) and cholesterol on log(TG) correlation constants give an indication of the frequency of hyperlipidemias

It can be noted that several investigators have found that type IIa is the most common form of hyperlipidemia followed by type IV (15 20 40) while we using arbitrary non age-dependent criteria find type IIa to be followed by type IIb (Table III) However not only the absolute but also the relative prevalence of different hyperlipidemia

Table VII *Dietary data for males by type of population*

Type of population	Energy (kcal/d)	Percentage of energy obtained as Fats	Saturated fatty acids	Carbohydrates
Lapps[a]	3 460	37	20	46
Rural Finns[b]	3 380	39	22	50
Semiurban Finns[b]	3 149	39	23	49
Finns employed in industry[b]	3 016	39	22	50

[a] Living in Finnish Lapland aged 20–59 (22)
[b] Aged 30–59 (47)

types in different sex age and population groups is affected by the choice of cut off limits For instance the apparent differences between middle aged men and women would be greatly augmented if the cholesterol cut off limit were raised to 320 mg/100 ml (8 28 mmol/l) Such a change would also make type IIb the least prevalent form of hyperlipidemia For all the sex and age groups combined the prevalences would then be type IIa 8 0% type IIb 6 8% and type IV 8 0%

Diet and lipid level Populations which obtain a large proportion of their dietary calories in the form of saturated fatty acids generally have high serum cholesterol concentrations (29) The fat consumption of various Finnish population groups (29 47) and the Lapps (22) is high and similar (Table VII) The similarities in diet might explain the similarities in serum lipid concentrations

Serum lipid levels and coronary heart disease Only part of the difference in CHD incidence and CHD mortality between populations can be explained by the classical risk indicators of CHD high serum cholesterol hypertension and smoking Thus Keys et al (29 31) reported that the observed CHD incidence in American men was higher than predicted from the levels of their risk factors and experience based on CHD incidence in European men In the same study the observed CHD incidence in East Finland was much higher than in the West and the difference was much larger than expected on the basis of the observed differences in risk factors Like Karvonen et al (26) we observe slightly higher cholesterol levels in East Finland than elsewhere in the country The finding of higher cholesterol levels in rural areas in the East below the age of 60 and lower levels thereafter might be caus

ally connected with a high CHD mortality rate in these rural areas

When searching for other risk factors the serum TG concentration has been considered Some prospective studies point to an association between initial TG levels and subsequent risk of CHD (9 11 45)

CHD incidence (29) CHD mortality rates and total mortality rates (8 13 34 51) differ considerably between various areas in Finland and between Finland and other Scandinavian countries Cholesterol and log(TG) levels on the other hand are quite similar in various populations in the area (Tables I and VI) It seems unlikely that differences in lipid levels of the magnitude observed could explain more than a small part of the variation in disease rates Follow up data in our populations will probably throw more light on this problem

It should be kept in mind that in Finland or in the parts of the country which have the highest CHD rates there could possibly be more individuals with high cholesterol or TG levels or both than would be expected on the basis of the population means or medians From the paper by Carlson and Lindstedt (12) we have calculated the age adjusted mean skewness of the cholesterol distribution in healthy non obese Swedes aged 30–59 years The values were 0 48 in men and 0 55 in women Values in unselected Finns were 0 72 in men and 0 71 in women Thus there is indeed some indication suggesting that skewness of the cholesterol distribution might be more marked in Finns The populations however are not fully comparable The possibility that what is perhaps the most hazardous combination of high cholesterol and high TG (11) is unusually common in Finns or in some Finnish population groups should also be explored Whereas it is possible that all types of hyperlipidemia are as common or more common in Finland than in other Scandinavian countries there was no evidence in our data that type IIb hyperlipidemia is more prevalent in East Finland where mortality rates are high

We must conclude that the reasons for the large differences in CHD mortality and incidence in Finland and the other Nordic area remain obscure Further standardized data on the prevalence of various types of hyperlipidemia in different population groups might yield additional clues Another possibility which may be more plausible is that the differences in CHD rates are largely caused by some still unidentified environmental or genetic factors

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Auscultatory Measurement of Blood Pressure Performed by the Doctor on Duty

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ABSTRACT A review is presented of the measurements of blood pressure on admission of 600 patients to the wards in a department of general medicine Three doctors each examined 200 patients, distributed over two periods with an interval of one year Large differences were found between the doctors as regards the distribution of the BPs, particularly the diastolic pressures On the other hand, no differences were observed in the distributions during the two periods as far as the individual doctors were concerned Great preference was shown for zero as the terminal digit and also for certain BP values It is concluded that the observer introduces a considerable subjective factor in measuring the BP by auscultation Correct performance of this simple procedure requires meticulous instruction

It has been demonstrated previously that in auscultatory measurement of the blood pressure psychological factors such as terminal digit preference and intermediate value bias are unconsciously introduced (2 3 4 5) In addition the interindividual variation in interpreting the Korotkoff sounds is well known (1) In these investigations however the individual observers have been aware that the measurements were to be used for research purposes It must be presumed therefore that they have been performed with care

It seems reasonable to investigate how routine measurements of blood pressure are carried out This we analysed in a department of general medicine

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METHOD

Doctors *A* *B* and *C* belonged to the privileged younger members of our profession who held a 2 year appointment in a University Clinic None of them had received special instruction in BP measurement and they were unaware of the problems attaching to terminal digit preference and intermediate value bias *A* had previously spent 2 years in another medical department *B* and *C* had after graduating been for 6 months and 2 years respectively in a surgical department

For each of the three doctors the study comprises 200 BP measurements recorded in the case notes viz the first 100 patients examined on admission by each doctor immediately after joining the staff of the Department (1st period) and another 100 patients whom they had examined after serving one year in the Department (2nd period) We also recorded the number of case notes in which BP was not stated by the admitting officer and of those in which only the systolic pressure was stated

The statistical analysis was performed by the rank sum test for comparison of two mean values (Mann Whitney s test) as well as by the χ^2 test

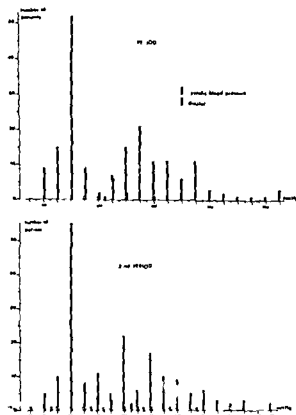
RESULTS

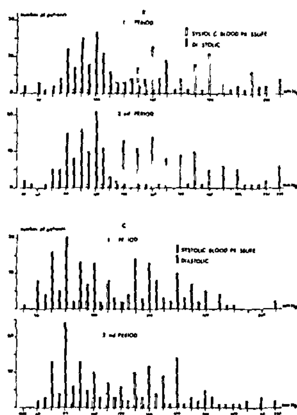
Normal preference

The number of 5s as the terminal digit of each of the three doctors 200 measurements is shown in Table I During the 1st as well as the 2nd period all three had a preference for 0 as the terminal digit This was particularly noticeable for *A* who had during the 1st period only one measurement ending on 5 *B* had a decreasing number of systolic BPs ending on 5 from the 1st to the 2nd period (χ^2 test $p<0\ 01$)

Distribution of the blood pressures measured

Fig 1 gives the distribution of the systolic and diastolic BPs





1 Systolic and diastolic BPs in 600 patients measured three doctors (A B and C) Each doctor admitted 100 patients during each of two periods at an interval of 1 year

Although the patients from the two periods were different the distributions for each observer were almost identical the only significant difference being between the distributions of diastolic pressures recorded by *B* ($p<0.05$ Table I) On the other hand there was a marked difference between the three observers the systolic pressures being grouped as follows *A* maximum at 120–130 mmHg *B* and *C* maximum at 140–150 mmHg Furthermore all three had another, not particularly well defined maximum at 160–180 mmHg

Greater and more striking differences were found in the diastolic pressures More than 50% of *A* s patients had diastolic pressures of 80 mmHg during both periods whereas the majority of *B* s patients had 90–100 mmHg *C* had a maximum at 80 mmHg although this was less striking than for *A*

Non recorded blood pressure

Table I lists the number of case notes which had to be perused for each of the two periods to procure 100 recorded BPs

From the 1st to the 2nd period there was for *A* and *B* an increase in the number of case notes in which the BP was not stated (χ^2 test $p<0.05$) whereas *C* had an increased number of case notes stating only the systolic pressure

DISCUSSION

From the above results it is apparent that BP measurement was performed differently by the three doctors All three had a striking preference for 0 as the terminal digit of the systolic as well as diastolic pressures *A* and *C* showed only a slight change in the number of 5s as the terminal digit from the 1st to the 2nd period whereas *B* had a marked decrease in the number of 5s as the terminal digit of the systolic pressures

The distribution of the diastolic pressures clearly showed that *A* had a pronounced peak at 80 mmHg whereas *B* had recorded the diastolic pressures as somewhat higher A partial explanation of

Table I *Results of the BP measurements on admission by doctors A B and C*

	Period	*A* Systolic	*A* Diastolic	*B* Systolic	*B* Diastolic	*C* Systolic	*C* Diastolic
BP mean±S D (mmHg)	1st	144 2	81 3	159 2	96 0	145 5	84 2
		28 6	14 1	34 9	18 2	27 7	15 6
	2nd	143 0	84 0	157 2	91 2	149 8	89 7
		30 1	15 0	33 8	14 4	31 2	19 1
Mann Whitney test							
t value		0 015	1 311	0 639	2 132	0 765	1 787
p		>0 1	>0 1	>0 1	<0 05	>0 1	>0 05
5 as terminal digit (%)	1st	1	0	24	39	30	19
	2nd	4	4	9	38	25	22
BP not recorded (no of pats)	1st	5		4		7	
	2nd	16		15		7	
Diastolic BP not recorded (no of pats)	1st	1		3		1	
	2nd	2		0		9	

the latter phenomenon is possibly that *B* when asked stated that he had always recorded the 1st diastolic BP (where the Korotkoff sounds grow muffled) whereas *A* had laid more emphasis on the 2nd diastolic BP (where the Korotkoff sounds cease)

With regard to the non recorded BPs all three doctors claim that they measured the BP on all admitted patients but that the values have not been recorded in the case notes

Since both periods comprise only 100 patients for each of the three doctors there is a theoretical possibility of a true difference between the patient groups On the other hand it is striking that the distribution of the BP levels of the patients of all three doctors had altered only very slightly from the 1st to the 2nd period Accordingly it must be assumed that the difference between the distributions is due to the observers This phenomenon was also noticed in a population BP survey in Bergen Norway (1) Prior to that screening study 19 nurses had been specially trained but nevertheless there were significant differences between the individual observers mean values

The main purpose of a BP measurement regarding the individual patient is to disclose whether he has too high too low or normal BP It would therefore seem of little significance whether the terminal digit is 0 or 5 On the other hand it may be of practical consequence if one doctor records the diastolic pressure as being 10–20 mmHg higher than another This applies to screening for hypertension as well as to the follow-up of patients on anti hypertensive therapy

CONCLUSION

On the basis of the present study—which attempts to assess the subjective factor in routine measurements of BP by three doctors who have probably not measured worse or better than most other doctors—we feel that we can conclude 1) Retrospective investigations of the relationship between routine BP measurements and other objective variables are of limited value This applies in particular if the measurements have been carried out by several different persons or by persons having no experience of this seemingly simple procedure 2) In prospective studies including BP measurements the individual observers need special instruction

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On the Use of Renal Angiography and Intravenous Urography in the Investigation of Renovascular Hypertension

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ABSTRACT The value of renal angiography for revealing renovascular changes has been investigated in 126 patients with hypertension, and in 102 of them a comparison was made with i v urography It was concluded that i v urography does not reveal renovascular changes and thus the primary investigation should be renal angiography, in some cases followed by urography in the same session

A large number of health surveys in different parts of the world have shown that the frequency of hypertension is high and Sweden is no exception Thus in screening programmes in the county of Uppsala during the last 10 years it has been found that 4% of men and 5% of the women above 16 years of age have hypertension (9) For examinations of 50 year-old men in the urban district of Uppsala a corresponding figure of 7 5% has been reported (8) Similar studies in Gothenburg have revealed the same high frequency of this disease (14 15)

High blood pressure is the dominating risk factor for the occurrence of cerebrovascular lesions and one of the main risk factors in coronary arterial disease This condition hypertension is therefore a great public health problem medically as well as economically

Patients with hypertension of a certain degree of severity must be investigated to exclude secondary hypertension The proportion of such cases is low perhaps under 3% among hypertensive patients in general (1) It is important however to diagnose secondary forms as radical treatment is available and chronic illness can be avoided In view of the high frequency of hypertension in the population an extensive investigation of hypertensive persons with the aim of detecting the few with secondary hypertension will place a great load on the health service system It is therefore necessary to make constant surveys of our investigation routines

An important group among patients with secondary hypertension is the group with the renovascular form The purpose of the present study was to find out what methods of diagnostic radiology are suitable for revealing renovascular disease thus to assess the value of intravenous urography as against renal angiography in this respect The roentgenological results were related to those of routine laboratory tests both in patients with roentgenologically diagnosed renovascular disease and in those with probable essential hypertension

This investigation seemed of special interest in view of current studies of preventive aspects in the Uppsala area and of our knowledge of a multicenter study of renovascular disease being carried out in the USA (3 4 12 13)

MATERIAL

During the period Jan 1970 – May 1972 inclusive renal angiography was performed on a total of 168 patients from the Department of Internal Medicine at the University Hospital in Uppsala Forty two of them were undergoing investigation for a suspected tumour or renal disease without hypertension Hypertension was the main reason for investigation in 126 patients (Table I) In 6 of them considerable impairment of the renal function apart from the hypertension was a contributory reason for the angiography During the period in question the indications for renal angiography in cases of hypertension were uniform and wide (2)

No i v urograms were available in 24 of the 126 hypertensive patients who underwent renal angiography The reason for this was that the urographies had been performed more than one year before the angiography in

Table I *Blood pressure mean age duration of hypertension serum creatinine and heart volume in 126 patients in whom the indication for renal angiography was hypertension*

BP[a]	♂/♀	Mean age (y) Total	♂	♀	Duration of hypertension (mo)	Serum creatinine (mg/100 ml)	HV (ml/m² BSA)	FH[b] grade	
191 (±29)/ 115 (±17)	71/55	45 0 (±12 2)	47 9 (±11 3)	42 5 (±11 2)	55 (±75)	1 48 (±1 52)	451 (±116)	0	34
								I	35
								II	38
								III	11
								IV	5

[a] The level at time of roentgenological work up
[b] Eyeground findings according to Keith Wagener & Barker S D within parentheses

question and were therefore not considered valid for comparison Urography was not carried out in a few further cases in whom renovascular disease was so strongly suspected that renal angiography was done directly or in patients in whom urography was considered meaningless due to greatly impaired renal function

METHODS

Intravenous urography The same technique was used in all patients 40 ml 60% Urografin® was injected i v into an arm vein About 3–4 min after the injection, compression as applied to the abdomen to prevent flow of contrast ium through the ureters to the urinary bladder The pelvis ureters and kidneys were evaluated with re t to their anatomy configuration and size Any delay excretion or difference in excretion between the two sides was noted

Renal angiography A catheter was inserted via the right or left femoral artery by the Seldinger technique A Grey Ödman catheter with one side hole and one end hole known as an Erikson catheter (5) was used In selective arteriographies 10 ml Angiografin® (306 mg J/ml) was injected and in aortography 30 ml A frontal projection of the aorta and exposures with the right and left sides respectively rotated about 15–20° were used The exposure frequency was 3 frames/sec for 4 sec followed by 1 frame/sec for 5 sec The injections were given with a Cisal II syringe 3 kp/cm² for selective injection and 5 kp/cm² for aortography ECG injection time and exposure were recorded simultaneously with a direct recorder All angiograms and urograms were inspected by two roentgenologists independently of one another

For evaluation of the grade of stenosis of the renal artery the following system was used Grade 0=no changes Grade I=only slight reduction of the lumen of the artery Grade II=reduction of the arterial diameter by less than 50% Grade III=reduction of the arterial diameter by more than 50% Grade IV=total occlusion In addition the configuration size and cortical layer of the kidneys were studied

Heart volume This was measured according to Jonsell (10) in erect or sitting position

Laboratory tests These included serum creatinine which was determined in an autoanalyser and urinary protein which was determined qualitatively with Albustix® and in the event of a positive finding qualitatively by an immunochemical method

Statistics Conventional methods were used for calculating mean values and standard deviations

RESULTS AND COMMENTS

Roentgenological findings

Both urography and renal angiography showed pathological conditions in 53 patients (Table II) Urography aroused suspicion of renovascular disease in only 2 of these patients in one of whom excretion was absent in a whole kidney and in the other in half a kidney This was found to be caused by total or practically total occlusion of the corresponding renal artery

In 22 patients the urograms were normal but the renal angiograms pathological In 21 of them the renal artery was constricted to varying degrees due to arteriosclerosis in 6 of these the stenosis was of grades III–IV In the remaining patient fibromuscular hyperplasia was present

Four patients showed pathological urograms but normal angiograms Three of them had calcifications in the urinary tract and one had ren mobilis

Normal findings both at urography and at renal angiography were made in 23 patients (Table III) In 18 of the patients for whom no comparative urograms were available the renal angiograms were pathological and stenosis of the renal artery was observed in 11 of them In 6 of the patients with no comparative urograms the renal angiograms were normal

The distribution of the different grades of stenosis among the patients is shown in Table IV Thirty-one patients had stenosis of grades I–II and 16 of grades III–IV (Table V) As seen in Table IV

Table II *Data on patients who underwent both urography and renal angiography and for whom the diagnoses of the two roentgenological methods were compared*

	No of pats	♂/♀	Mean age (y) Total	♂	♀	Duration of hypertension (mo)	Serum creatinine (mg/100 ml)	HV (ml/m² BSA)	FH	grade
Pathological urography/pathological angiography	53	31/22	45 7 (±13 4)	48 6 (±13 7)	41 5 (±12 1)	57 (±83)	1.55 (±1 37)	471 (±127)	0	17
									I	16
									II	13
									III	4
									IV	2
Normal urography/pathological angiography	22	11/11	47 9 (±11.5)	50 5 (±8 8)	45 3 (±13 5)	64 (±98)	1 09 (±0 39)	463 (±118)	0	1
									I	8
									II	9
									III	[illegible]
									IV	[illegible]
Pathological urography/normal angiography	4	2/2	46 3 (±9 2)	52 5 (±5 0)	40 0 (±8.5)	8 (±10)	1.20 (±0 41)	368 (±120)	0	2
									I	0
									II	1
									III	1
									IV	0
Normal urography/normal angiography	23	14/9	43 0 (±7 7)	43 7 (±7 3)	41 8 (±8 7)	51 (±60)	0 94 (±0 15)	394 (±68)	0	9
									I	8
									II	5
									III	0
									IV	1
Urography not performed/pathological angiography	18	9/9	47 2 (±12 2)	50 1 (±10 5)	43 6 (±13 3)	36 (±34)	2 56 (±2 97)	464 (±116)	0	1
									I	3
									II	9
									III	2
									IV	2
Urography not performed/normal angiography	6	4/2	42 0 (±8 9)	40 8 (±11 0)	44 5 (±3 5)	69 (±58)	1 45 (±1 06)	450 (±67)	0	4
									I	0
									II	1
									III	1
									IV	0

Eyeground findings   S D within parentheses

no fewer than 3 patients had renal cancer The heart volume lay within normal limits for both men and women but there was a tendency towards a somewhat higher mean value in the group with both pathological urograms and pathological angiograms

Laboratory tests in different roentgenological groups

In some of the subgroups (Table II) the serum creatinine values were raised This was not unexpected in the two groups of patients who did not undergo urography as in some cases an elevated serum creatinine value was the reason why urography was not performed These cases explain the differences in mean values In the large group in which both the urograms and the angiograms were pathological the increased mean value could be explained by patients who in addition to their renal arterial stenosis also had another kidney disease

Of the 53 patients in the latter group only 7 had no other signs of kidney disease These 7 had no proteinuria no raised serum creatinine and no positive history of urinary tract infections Six of these had minor congenital anomalies and one patient had unilateral hydronephrosis which also was seen on the angiogram

In the group with no comparative urograms but with pathological angiograms fairly advanced hypertensive changes on the eyegrounds were observed This finding probably reflected relatively long delays in the investigation procedures—often the patient had been referred from another hospital The suspicion of renovascular involvement was strong in this selected group and for this reason angiography was often performed directly

Table III *Relation between normal and pathological findings in i v urography and renal angiography*

Urographic diagnosis	Angiographic diagnosis					
		Pathologic				
		Stenosis				
	Normal	I–II	III–IV	Varia	Total
Normal	23	15	6	1	45
Pathological	4	8	7	38	57
Urography not performed*	6	8	3	7	24
Total	33	31	16	46	126

* *Usually because of high level of serum creatinine*

No statistically significant difference was found between patients with renal arterial stenosis of grades I–II and those with grades III–IV with respect to age duration of hypertension or heart volume (*Table V*) *Worthy of note was the difference* in serum creatinine concentration between the groups with different grades of stenosis The group with ologically *milder* stenosis had a higher serum creatinine concentration This was ibly because some of *these* patients had a dis of the renal parenchyma causing their hypertension

Radiorenography was performed in only 23 patients One noteworthy observation was that in 12 of the patients with stenosis of grades I–II at angiography the radiorenogram was normal while in 6 of 11 patients with stenosis of grades III–IV it was pathological (Table V)

DISCUSSION

One of the *principles* followed in our hospital in the investigation of hypertensive patients during the period in question was that both patients with a relatively rapid onset of hypertension and those with known previously mildly treated hypertension *in whom the treatment had failed* were investigated for renovascular hypertension The present series is thus representative of the types of patients who in this country are usually investigated for suspected renovascular disease The material is not comparable *in size with the cooperative* material reported from the USA (12) but has the advantages of a more uniform evaluation and standardized methods both with respect to roentgenological examination and to laboratory tests of blood and urine Men predominated in this series and they had a higher mean age than the women *This is not unexpected in view of the fact that* hypertension in men *is* always more serious than in women at the same pressure level (*11*) and that men therefore predominate as investigation cases

When investigating suspected renovascular hypertension urography alone *is* not sufficient In our experience the rapid sequence technique yields no definite improvement of the diagnosis in urography It is also reported from the cooperative study in the USA that among patients who underwent operation and were cured of their renovascular disease the preoperative rapid sequence urography was normal in about 10% (3) In another study the corresponding result was even worse with 31% false negative findings (7)

Of 22 patients with normal urograms in our series *renal* arterial stenosis was observed at renal arteriography *in 21* cases 6 of them of grades III–IV and fibromuscular hyperplasia in one case (Table III) As complete renin tests were not performed on the entire series we cannot on the other hand definitely state that these roentgenological *stenoses represented renovascular hypertension* pathophysiologically Further *it* should be pointed out that in the group with pathological urograms (57 patients) only 2 patients showed changes at urography which aroused suspicion of vascular involvement—*in the form of absent excretion in*

Table IV *Diagnoses in patients with both pathological urography and renal angiography*

Diagnoses	No of pats
Pyelonephritis	16
Cysts single or multiple	9
Hypoplasia	4
Renal cancer	3
Hydronephrosis	2
Concrement*	3
Anomalies*	6
No excretion	
(one kidney)	1
(one half of a kidney)	1
Others	8

* One patient had both concrement and hydronephrosis and another concrement and cysts
* *Include* duplicature of pelvis foetal lobulation horse shoe kidney etc
ᶜ Include pyelogenic cyst transplanted kidney etc

Table V *Relation between the different grades of renal arterial stenosis and age duration of hyper tension serum creatinine heart volume eye ground changes and radiorenographic findings*

	Grade of stenosis	
	I–II	III–IV
n	31	16
♂/♀	16/15	9/7
Mean age (y)		
Total	50 2 (±8 8)	48 5 (±11 5)
♂	52 6 (±8 5)	53 4 (±10 6)
♀	47 7 (±8 6)	41 0 (±9 1)
Duration of hyper tension (mo)	55 (±62)	63 (±81)
Serum creatinine (mg/100 ml)	1 43 (±1 49)	1 15 (±0 34)
HV (ml/m² BSA)	460 (±80)	488 (±102)
FH grade		
0	2	2
I	7	5
II	16	8
III	5	1
IV	1	0
Radiorenogram	12 normal/ 12 performed	5 normal and 6 pathological/ 11 performed

Eyeground findings S D within parentheses

half of one kidney in one case and in a whole kid ney in the other The underlying cause of this was an angiographically observed renal arterial stenosis of grade IV As mentioned previously in only 4 pa tients were the urograms pathological and the renal angiograms normal In all of these cases the findings were probably of no importance for the hyperten sion

A normal i v urogram does not exclude the pres ence of renal arterial stenosis as is evident from Table III Furthermore in the group of hyperten sive patients with pathological urograms the fre quency of renal arterial stenosis was possibly lower than in the group with normal urograms The reason for this might be that the other pathological condi tions may be associated with the hypertensive dis ease This could thus mean that in one group of patients the hypertension is of renovascular origin alone due to constriction of the renal artery while in another group it is caused by changes in the renal parenchyma From this line of reasoning it seems motivated to continue a renal angiography with a urography i e to utilize the contrast medium that has already been injected It is probably also of value to add videodensitometry for estimation of the circulation time and regional blood flow

The patients with renal arterial stenosis of grades III–IV (in which group renovascular hypertension is probably mainly to be found) cannot be distin guished clinically from the group with stenosis of grades I–II with respect to age duration of hypertension and heart volume The surgical tr at ment of renal arterial stenosis can vary and is obviously affects the indication for renal angiogra phy In our series 8 of 16 patients with steno s of grades III–IV underwent operation

It is true that the duration of hypertension prior to renal angiography in this series may reflect our at titude towards the indications for this examination as is partly illustrated by the fact that the duration in our patients was longer than in the material from the USA referred to above (12) In the renal hyperten sion due to atherosclerosis in the American cooperative study the duration of the clinically diagnosed hypertension was thus 1 9 years while in our series it was 5 3 years at the same time the mean age in the two series was in good agreement i e 49 7 and 48 5 years

It would therefore seem appropriate—instead of considering the duration age serum creatinine value and heart volume—to take into account the course of the disease and to continue with renal angiography only in patients who are initially re fractory to hypertension therapy and those who have become therapy resistant

In view of the great value of renal angiography it is important to know the complication frequency as an increase in the number of examinations might conceivably increase the number of complications *both in renal arteries and at the site of puncture* as in all other angiographies The risk of complications has been analysed in a larger series with respect to the renal artery and has been found remarkably low (6)

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Changes in Plasma and Extracellular Fluid Volume during Long-term Treatment with α-methyldopa in 11 Thiazide-treated Patients with Essential Hypertension

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ABSTRACT In eleven patients with essential hypertension, plasma volume (T 1824) (PV), extracellular fluid volume ($^{82}Br^{-}$distribution space) (ECFV) blood pressure (BP) and the ratio plasma to interstitial fluid volume (PV/IF) have been determined three times before treatment, after more than two months of thiazide treatment and after three and six months of combined thiazide–α methyldopa (AMD) therapy During combined treatment PV/IF showed a significant increase whereas PV and ECFV did not change significantly Compared to values during thiazide therapy, PV and PV/IF increased, whereas ECFV was unchanged BP was reduced significantly after thiazide treatment and showed a further significant fall when AMD was added Based on the results and a review of the literature it is concluded that AMD acts by interfering with the normal sympathetic activity, thereby producing a reduced venomotor tone and increased venous capacity The concomitant reduced venous return of blood to the heart is suggested to contribute to the antihypertensive effect of AMD by means of a decreased cardiac output

Most potent antihypertensive drugs are likely to cause considerable fluid retention and this has been proved in some cases to inhibit the effect of the treatment (5) This does not apply to diuretics and β blockers (16 23) Some authors have maintained that long term treatment with α methyldopa (AMD) might also cause fluid retention This theory is based mainly on changes observed in body weight during treatment and occasional development of oedema (1 8 15) Only a few determinations of body fluid volumes before and during long term treatment with AMD are available (1 9)

The object of the present study was to elucidate the effect of long term AMD treatment on plasma volume (PV) extracellular fluid volume (ECFV) body weight and blood pressure (BP) in patients with essential hypertension who were treated with thiazide over several months

MATERIAL AND METHODS

The group studied comprised 11 patients with essential hypertension selected at random from those visiting our Outpatient Clinic for Hypertensives None of them had signs of congestive heart failure and they did not suffer from diseases known to influence the parameters investigated The clinical data appear in Table I

All examinations were carried out at 8 a m The patients had had nothing to eat or drink for 9 hours and had rested in bed for at least 1 hour

PV was determined with T 1824 (Evans blue) and ECFV was measured as the distribution volume of ^{82}Br as previously described (16 17) Interstitial fluid volume (IF) was calculated as ECFV–PV BP was recorded by applying an arm cuff and mercury manometer Each value represents the average of three consecutive determinations after at least 1 hour s rest in bed The mean BP (MBP) was calculated as the diastolic BP plus one third of the pulse amplitude Before institution of treatment PV and ECFV were determined three times at 1 week intervals The averages of these determinations were used as control levels and appear in Table I

The patients were given 75–100 mg (mean 77) hydrochlorothiazide daily Two patients (nos 1 and 2) received *supplementary potassium chloride*

Other antihypertensive drugs were given to 4 patients for a few months and then withdrawn so that all patients were treated solely with hydrochlorothiazide for at least 2 months The medication was supplemented with AMD in doses sufficient to control the BP (mean 1 160 range 750–1 750 mg) No other drugs were given during the investigation period

The determinations of PV and ECFV were repeated

Table I *Clinical data of 11 patients with essential hypertension prior to antihypertensive treatment*

Case no	Age (y)	Sex	Height (cm)	Weight (kg)	BP* (mmHg)	S-creatinine (mg/100 ml)	FH•	Control values PV (ml)	Control values ECFV (ml)
1	68	♂	166	83 2	217/132	1 0	II	3 619	19 344
2	60	♀	162	47 4	207/118	1 3	I–II	2 239	12 651
3	54	♂	173	76 6	203/125	1 5	II	4 254	20 600
4	57	♀	166	68 8	207/123	0 9	I–II	3 480	17 780
5	48	♂	171	83 4	178/115	1 0	I	3 799	18 697
6	59	♀	158	78 7	223/120	0 9	II	2 760	16 248
7	55	♂	173	66 3	230/135	1 6	III	3 120	15 674
8	49	♂	169	80 2	183/127	1 1	I–II	3 081	18 638
9	66	♂	178	77 5	210/123	1 1	II	3 719	18 127
10	47	♀	152	47 2	183/110	1 1	I	1 869	10 655
11	55	♂	171	71 3	210/127	1 4	II	2 614	15 309

Average of three determinations
• Eyeground change graded according to Keith Wagener

after at least 2 months of treatment with thiazide only and after 3 and 6 months of combined thiazide/AMD therapy Changes in the parameters investigated were calculated as differences between the averages of the three control levels and the values determined after 2 months on thiazide and 3 and 6 months of combined treatment respectively Statistical calculations were made applying Student s *t* test for paired differences

RESULTS

Table II shows changes compared to control values The PV/IF ratio increased significantly after both 3 and 6 months of combined therapy PV tended to increase but significantly only at the 10% level after 3 months of combined therapy ECFV did not change significantly (Table II)

Table III shows changes compared to values during thiazide treatment PV increased significantly after both 3 and 6 months of treatment The PV/IF ratio tended to increase after 3 months and was significantly higher after 6 months of combined therapy ECFV did not change significantly (Table III) The changes in body weight were insignificant and no general tendency or correlation between body weight and changes in plasma volume or ECFV was observed The average increase was +1 kg (range −3 8 to +4 2) and +1 9 kg (range −4 2 to +5 8) after 3 and 6 months of combined therapy Compared with thiazide treatment AMD caused an average change in body weight of +0 7 kg (range −3 8 to +4 6) and +1 6 kg (range −4 2 to +7 0) after combined treatment for 3 and 6 months respectively

MBP was reduced significantly both after 3 months of combined treatment (mean −31 mmHg S D 8 0 $p<0\ 001$ n 9) and after 6 months (mean −31 mmHg S D 11 9 $p<0\ 001$ n 10) When AMD was added to the thiazide treatment a further significant reduction in MBP occurred both after 3 months of combined therapy (mean −15 mmHg S D 13 8 $p<0\ 02$ n 8) and after 6 months (mean −16 mmHg S D 14 3 $p<0\ 02$ n 9) No differences in the MBP reduction were observed between combined therapy for periods of 3 and 6 months

Table II *Changes in plasma and extracellular fluid volume and the ratio plasma to interstitial fluid volume after 3 and 6 months of combined thiazide–α methyldopa treatment*

	Thiazide and AMD 3 mo (9 pats)	Thiazide and AMD 6 mo (10 pats)
PV (ml)		
Mean	+127	+92
S D	176	183
S E M	59	58
p	0 05<*p*<0 1	0 1<*p*<0 2
ECFV (ml)		
Mean	−217	−38
S D	730	707
S E M	244	224
p	>0 3	>0 8
PV/IF		
Mean	+0 0176	+0 0101
S D	0 0227	0 0127
S E M	0 0075	0 0040
p	<0 05	<0 05

Table III *Changes in plasma and extracellular fluid volume and the ratio plasma to interstitial fluid volume after 3 and 6 months of combined thiazide–α methyldopa treatment compared to values during thiazide therapy alone*

	Thiazide and AMD	
	3 mo (8 pats)	6 mo (9 pats)
PV (ml)		
Mean	+189	+200
S D	225	133
S E M	80	44
p	<0 05	<0 005
ECFV (ml)		
Mean	+68	+250
S D	551	613
S E M	195	204
p	>0 7	>0 3
PV/IF		
Mean	0 0188	0 0149
S D	0 0268	0 0162
S E M	0 0094	0 0054
p	0 05<*p*<0 1	<0 05

DISCUSSION

The present investigation revealed changes in equilibrium between PV and IF and increased PV during long term treatment with AMD Other authors have found increased BV both during short term and long term trials with AMD alone (1 9) These observations together with observed increases in body weight and occasional development of oedema in a few cases during treatment with AMD have been considered to support the theory of universal fluid retention (1 4 8 15) Development of oedema will occur most often in patients with a very high diastolic BP or impaired renal function and is most likely associated with slight cardiac failure None of our patients presented significant increases in body weight ECFV remained unchanged and no correlation could be demonstrated between changes in body weight and ECFV

The increase in PV averaged 6% A corresponding increase in ECFV would be 1000 ml The 99% confidence limits for changes in ECFV prove that such a high increase in ECFV could not have been overlooked This is further confirmed by the fact that the PV/IF ratio also increased significantly as an expression of changed equilibrium between PV and IF These results agree with the findings by Hansen (9)

Thus in patients with essential hypertension without renal or cardiac complications no general fluid retention will occur when long term thiazide therapy is combined with AMD During long term trials changes in body weight cannot be used as an indication of changes in the body fluid volume

The antihypertensive effect of AMD is well established (8 15) and was also found in the present study The antihypertensive mechanism is not fully clarified Patients with essential hypertension have increased sympathetic activity (7) which might explain why the venous part of the circulatory system also participates in the overall increase in peripheral resistance (2 3 25) It seems to be proved that AMD inhibits the function of the sympathetic nervous system both centrally and in the peripheral nerve ends (10 11 13 14 15) although the nature of the haemodynamic effect remains unclear Some authors found reduced peripheral resistance during long term trials whereas others demonstrated reduced cardiac output associated with unchanged peripheral resistance (18 21)

In untreated patients with essential hypertension the PV/IF ratio is significantly lower than in normotensive individuals (12 22 24) During long term thiazide therapy this abnormal pattern is maintained The increased PV unchanged ECFV and the changed equilibrium between PV and IF during long term treatment with AMD might be explained by reduced venous tone resulting in increased venous capacity and reduced hydrostatic pressure in the venous end of the capillaries The result will be reduced venous return to the heart and reduced cardiac output which might contribute to the antihypertensive effect of AMD This was also suggested by Mason and Braunwald (19) who proved that AMD is able to block the reflex venoconstriction in normotensive individuals

Other authors have proved that reduced effect of antihypertensive therapy is often associated with increased intravascular volume and that these conditions can be corrected by intensified diuretic therapy (5) In the present study PV and ECFV were found to be suitably controlled by combined thiazide–AMD therapy The slight increase in PV following institution of AMD treatment supports the theory that measures should be taken to correct any possible failing effect of antihypertensive treatment by increasing the dose of diuretics

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A Trial of a New Adrenergic β-receptor Blocker, ICI 66 082, in the Treatment of Hypertension

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ABSTRACT From a survey of 1250 males, 40–60 years of age, 25 mildly to moderately hypertensive patients have been offered treatment with the new β blocker ICI 66 082 Before and during the trial data were obtained on resting BP and pulse rate BP, pulse rate and ECGs during near maximal exercise, and various ventilatory function tests In 15 patients, satisfactory BP control was obtained with ICI 66 082 alone, 25–100 mg twice daily In 5 patients, the addition of hydralazine or a diuretic was necessary for satisfactory response In 2 patients satisfactory control was not achieved and 3 patients were excluded for various reasons Although BP and maximal pulse rate fell markedly during exercise the total work capacity was not significantly reduced No deleterious effect on ventilatory function was noted

In recent years it has become widely accepted that adrenergic β receptor blockers (β blockers) possess potent antihypertensive properties (8 13 18) Research in this field aims at making a drug free from deleterious effects on the ventilatory function and *the peripheral vessels A new β blocker must also* compare favourably with existing β blockers with regard to short and long term potency side-effects dosage schedules etc

The new β blocker ICI 66 082 may represent such an advance in β blocker therapy Animal experiments and limited human experience show that the drug has potent β blocking properties acts almost exclusively on the β_1 receptors and only to a very limited extent on the β_2 receptors in the bronchi and peripheral vessels (2 6 7 17) It has no intrinsic β stimulatory effect and no cardiodepressive actions in the reserpinized animal (10) Serious side-effects have not been reported and the serum half life of 5 hours suggests a dosage twice daily (5 9)

The present study was undertaken to evaluate this new β blocker in the treatment of formerly untreated essential hypertension of mild and moderate severity

MATERIAL AND METHODS

In an epidemiological survey of males between 40 and 60 years of age conducted at our department subjects with hypertension were offered therapy All individuals with a mean diastolic BP above 105 mmHg on 3 occasions at least 2 weeks apart (3 measurements on each occasion) have been included BP measurements were made to the nearest 2 mmHg below according to the technique of Rose and Blackburn (15) phase 1 being taken as the systolic and phase 5 as the diastolic pressure Individuals with known or suspected heart disease have been excluded from the survey as well as individuals with diabetes mellitus malignancy or disorders of the locomotor system preventing a near maximal bicycle exercise test

Among the first 1250 individuals 25 were hypertensive according to these criteria Of these 3 have been excluded from the trial one because of poor cooperation one because of side-effects and one because he refused to participate The mean age of the treatment group was 49 6 years (S D 5 0) mean height 168 0 cm (S D 7 3) and mean weight 78 4 kg (S D 10 0) The mean age was similar to that for the total material while height was significantly lower and weight significantly higher

Before and during therapy BP was measured with the subjects sitting (BP I=casual BP) sitting during relaxed expiration after a full inspiration (BP II) and after 5 min rest in the supine position (BP III)

Before treatment was started a resting ECG was taken and a near maximal exercise test with inexercise and post exercise ECG recordings was performed The exercise test was performed on an electrically braked bicycle the ECGs were taken with a Mingograph 61 ECG recorder Twelve lead ECGs were taken before and after exercise while chest head leads were used during exercise The starting load was 600 kpm the load being increased every 6th min by 300 kpm The exercise was continued until at least 90% of the age predicted heart rate had been (14) or until fatigue or exhaustion or signs

Table I *Relation between resting pulse rate and effect of treatment*

++ = Primary and secondary good/excellent effect +− = primary good/excellent effect with secondary rise during the first weeks/months of therapy −− = primary and secondary therapeutic failure +−+ = +− with a long term good/excellent effect of ICI 66 082 + hydralazine or a diuretic +−− = +− with negligible effect of additional therapy −−− = −− with (at present) no effect of other therapeutic agents used

Pulse rate	No of pats	Primary/secondary anti hypertensive effect ++	+−	−−	Effect of additional therapy +−+	+−−	−−−
<60	3	2	1			1	
60–69	5	3	2		2		
70–79	8	4	3	1	3		1
80–89	3	3					
90–99	2	2					
≥100	1	1					
Total	22	15	6	1	5	1	1

discontinuation of the test occurred During the test ECGs were recorded at 1/2 1 2 4 and 6 min during the first load and at 2 4 and 6 min on all other loads BP was measured at $5^1/_2$ min during the first load at $3^1/_2$ and $5^1/_2$ min during the second load and at $1^1/_2$ $3^1/_2$ and $5^1/_2$ min during all other loads Following exercise ECGs were recorded continuously sitting on the bicycle during the first $^1/_2$ min and later supine at 1 2 3 and 5 min When signs of ischemia were found the ECG was followed for 10 min

During treatment an identical exercise test was performed after at least 3 months therapy The tests were all performed between 6 and 8 hours after the morning e The ECGs have been coded according to the ndinavian modification of the Minnesota code (16)

Spirometric measurements were made before and after at least 3 months of therapy Vital capacity and 1 0" forced expiratory volume (FEV 1 0") were determined with a Bernstein spirometer peak expiratory flow (PEF) was measured with a Wright s peak flowmeter the mean value of the last 3 of 5 consecutive determinations being used

The starting dose of ICI 66 082 was 25 mg twice daily increments were made after at least one month on the same dose The effect was evaluated every fortnight during the first 2 months and later every 2–3 months A fall in BP to below 140/90 mmHg was considered satisfactory If the primary response to ICI 66 082 was poor i e if values of 150/100 or above were recorded or if a secondary rise in BP occurred hydralazine (Apresoline 25 mg three times daily) or a diuretic was added to the regimen When combined treatment was given the dose of ICI 66 082 was always 50 mg twice daily Dosages higher than 200 mg twice daily have not been used On each visit the patients were specifically asked for side-effects

RESULTS

During treatment the BP dropped to below 140/90 in 12, to 140/90–150/100 in 6 and stayed consistently above 150/100 mmHg in 4 patients In 1 case BP failed to drop at least 10 mmHg and in 6 cases there was a secondary rise to unsatisfactory levels These 7 individuals have been put on additional therapy 5 on hydralazine and 2 on a diuretic In only 2 of these cases was combined therapy insufficient to bring the BP to values below 150/100 mmHg

The resting pulse rate dropped from 72 6 to 60 3 but as it was only 59 in the total group of 1250 individuals it seems that the hypertensives have a high resting pulse rate There was no correlation between drop in pulse rate during treatment and the BP lowering effect although there was a tendency towards a better effect in individuals with high initial pulse rates (Table I) Noteworthy however are 2 individuals with initial pulse rates of 52 and 57 who had BP reductions during treatment of 54/30 and 30/18 mmHg respectively without a con

Table II *Effect of treatment versus dose of ICI 66 082*

Maximal ICI 66 082 dose (mg)	No of pats	Mean pre treatment BP	Mean treatment BP (or BP at the time of additional therapy)
25×2	7	165 5/110 6	134 0/91 0
50×2	6	173/115 3	135/89 7
100×2	6	176/119 7	163/103 7
200×2	3	170/115	178/108
50×2+ hydralazine 25×3	5	175 5/117 5	141 2/92 5
50×2+ diuretic	2	168 5/112 2	147 5/94 6

Table III *Spirographic data before and during treatment with ICI 66 082*

	Before	S D	During	S D
PEF (l/min)	511	78	548	69 $p<0\ 05$
FEV 1 0" (ml)	3 402	525	3 363	504 $p>0\ 10$
Vital capacity (ml)	4 127	582	4 200	564 $p>0\ 10$

comitant drop in pulse rate None of the individuals with a drop to below 140/90 during treatment showed a secondary rise in BP while 3 with a BP drop to 140/90–150/100 mmHg showed a secondary rise in the early treatment period

Table II shows the effect in relation to the dose of ICI 66 082 The highest dose has been 200 mg twice a day though none use this dose at present The pretreatment BP is similar in all dosage groups but the effect is best with the lowest dosages This suggests that if a satisfactory effect is not obtained with the low dose a further increase of dosage will not yield an additional effect

No changes were found regarding respiratory function during treatment (Table III) During near maximal exercise the systolic BP was on an average 42 mmHg lower on therapy (Table IV) The same Table also shows that the maximal pulse rate obtained during pretreatment and treatment was 163 8 and 127 0 beats/min respectively The maximal product of systolic BP and pulse rate fell from 41 580 to 28 027 while the cumulative work only dropped from 10 540 to 9 586 kpm The cumulative work dropped in 8 was unchanged in 9 and increased in 5 patients

The individuals with increased physical performance were mainly those with high initial pulse rates The mean drop in cumulative work did not reach statistical significance

ECGs taken during exercise showed considerable normalization during treatment (Table V) In 4 individuals coronary angiography has been performed Three of them had atherosclerotic changes of one or more coronary artery and one had a normal coronary angiogram These individuals too showed normalization of the exercise ECG

Side effects were few One subject withdrew from the trial because of insomnia and nightmares and has been excluded Two complained spontaneously of cold hands while most of the others described similar complaints on inquiry This side

Table IV *Data from near maximal exercise test (n=22)*

A=before B during therapy with ICI 66 082
Figures within parentheses=S D

Resting pulse rate (beats/min)	A	72 6	(12 4)
	B	60 3	(7 3)
Maximal pulse rate during exercise (beats/min)	A	163 8	(18)
	B	127 0	(20)
Maximal systolic BP during exercise (mmHg)	A	251	(14)*
	B	209	(20)
Maximal product of pulse ×systolic BP	A	41 580	(4 512)*
	B	28 027	(5 570)
Cumulative work during test (kpm)	A	10 540	(3 500)**
	B	9 586	(3 403)

(A–B) difference statistically significant at $p<0\ 001$
* not statistically significant

effect however did not necessitate withdrawal even during winter time Approximately one half complained of initial tiredness and fatigue of the lower limbs but this tended to disappear as therapy continued Renal function followed by serum creatinine determinations did not change during therapy

DISCUSSION

The first report of β adrenergic blockers in the treatment of hypertension dates from 1964 (12) and their use is now well established At present β blockers are often used as the first drug in the treatment of hypertension except when definite contraindications exist The combination of β block

Table V *ECG coding before and during therapy with ICI 66 082 (near maximal exercise test)*

Minnesota code	No of pats: Before treatment	During treatment
4 0	9	18
4 7	1	
4 6	2	1
4 3	1	
4 2 s	0	
4 2 i	2	2
4 1 s	7*	1

* 4 underwent coronary angiography 3 with positive angiograms 1 with negative angiogram 2 with angiography+had 4 0 on the exercise test during therapy with ICI 66 082 1 had 4 2 i-response The individual with negative angiogram had 4 2 i response

ers and vasodilators in more resistant cases seems to be particularly favourable (11)

Today a large number of potent β blockers are available Theoretically however ICI 66 082 has possible advantages Animal experiments indicate that it is a highly selective β_1 blocker i e it has little or no effect on the β receptors of bronchial muscle and peripheral vessels (6 7 10) No intrinsic β stimulatory effect seems to be present nor has a membrane stabilizing effect been described (quinidine-like effect) (10)

The present study indicates that the antihypertensive effect of ICI 66 082 is rapid and of considerable magnitude in individuals with a mild or moderate hypertension In some individuals however there is a primary drop in BP followed by a secondary rise to pretreatment levels In most of these cases combined treatment with hydralazine (3 cases) or a diuretic (2 cases) has proved satisfactory The results indicate that a beneficial effect is often seen with very small dosages such as 25 mg×2 In fact if small dosages are not effective in lowering the BP an increase up to 200 mg×2 has not proved effective

Amery et al (1) found no correlation between the fall in BP on this drug and the initial peripheral or initial mean BP We have never used dosages but the results indicate that a increase of the dosage is unwarranted This is in contrast to what seems to be the case with propranolol (13 18) The data are limited but they also indicate that one might expect a better effect in individuals with high pulse rates than with low In the individual case however one cannot predict the effect of treatment from the pretreatment pulse rate

As was expected the effect on the BP during maximal exercise was even more marked than at rest It is therefore remarkable that the mean cumulative work achieved was only insignificantly decreased during treatment with ICI 66 082 On the drug 5 patients increased their cumulative work 8 had a decreased performance and 9 had identical values

As with other β blockers ischemic ECG changes during work were normalized during therapy with ICI 66 082 The results indicate that ICI 66 092 may be of value in the treatment of angina pectoris However, none of our patients had angina pectoris

The spirographic data show that the drug has a negligible influence on the bronchial muscles this has been shown in labile asthmatics as well (17) In our study however no individual had decreased ventilatory function beforehand The small increase in peak flow values found during therapy is probably explained by a learning effect even though at least 3 months lapsed between the two measurements

As with most β blockers serious side-effects were few (8 13 18) However cold hands muscular weakness and general fatigue occurred fairly regularly during the initiation of therapy but tended to disappear as therapy continued The only side effect that necessitated withdrawal was nightmares experienced by 1 patient on the evening he started with ICI 66 082 This effect has also been reported with other β blockers

ICI 66 082 has a half life in blood of 5–6 hours (5) One should be cautious however when drawing definite conclusions about the dosage schedules from pharmacokinetic studies Propranolol for instance has a half life of about 3 hours yet it may be given twice daily in the treatment of hypertension (3 4) In this study ICI 66 092 has been given twice daily all tests being made 6–8 hours after the morning dose The results therefore indicate that this may be a suitable dosage schedule although one dose a day may be worth trying

In 13 individuals the effective daily dose was 50 mg×2 or less Patients in whom we had to increase the dose to 200 mg×2 invariably had to change to a combined treatment to obtain satisfactory BP reduction The manufacturer s suggestion that 100 mg×2 should be the starting dose thus seems unwarranted On the contrary 25 mg×2 seems more appropriate at least in cases of mild to moderate hypertension

Our patients were unselected hypertensives taken from an epidemiological survey of presumably healthy males aged 40–60 years It is therefore safe to assume that ICI 66 092 will give a satisfactory response in most cases of mild hypertension It compares favourably with other antihypertensive drugs in this respect

No comparative study or cross-over study has been performed nor has a double blind technique been used The study therefore is inconclusive as to whether this drug should be preferred but indicates that more and better studies should be performed to resolve this question Theoretically however the negligible effect on the bronchi and peripheral vessels represents an advantage and may indicate a

future for this drug in the treatment of hyperten sion The reason for the secondary BP rise in a particular case must be explained from long term haemodynamic studies The mode of action of βblockers in hypertension is still widely debated

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Studies in Asymptomatic Primary Hyperlipidaemia

IV *ECG at Rest and during Exercise and Its Relation to Various Lipoprotein Classes*

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ABSTRACT Among approximately 20000 apparently healthy subjects whose serum cholesterol and triglyceride (TG) values were screened at a health examination, those with the most pronounced hyperlipidaemia have been selected for further studies Thus 188 males and 126 females aged 30–65 years, with asymptomatic primary hyperlipoproteinaemia (HLP) and 59 male and 69 female controls with non-elevated serum lipids were studied with regard to frequency of ST segment depressions during exercise to near maximal heart rate Furthermore, the concentrations of cholesterol and TG were determined in the lipoprotein (LP) classes very low (VLDL), low (LDL) and high (HDL) density LP separated by preparative ultracentrifugation From the LP analysis each subject's HLP was classified according to the typing system of HLP recommended by the WHO The frequency of ST depressions (Minnesota code 4 1–4.3 as well as 4 1–4 4) increased with age was higher in females than in males and was increased in all types of HLP in males The percentage frequencies of ST depressions 4 1–4 4 in the various types of HLP were (male/female, p against controls) controls 16/36, type II A 50 ($p<0$ 01)/56 type II B 64 ($p<0$ 01)/75 ($p<0$ 05) type III 67 ($p<$ 0 01)/33 and type IV HLP 40 ($p<0$ 01)/53 There was no significant difference in the frequencies of ST depressions in subjects with "high" and "low" BP (hypertensives were excluded from the study) or in subjects with "high and low k value for the i v glucose tolerance Non smokers had a tendency to higher frequencies of ST depressions than smokers The association between different LPs and other risk factors' and the occurrence of ST depressions in HLP were studied further with multiple regression analysis Invariably age was the best predictor of ST depressions The LP fraction giving the highest correlation coefficient was LDL cholesterol in both sexes VLDL TG and LDL TG were also positively and significantly associated with ST depressions HDL cholesterol was negatively but insignificantly correlated to ST depressions When age and LDL cholesterol had been entered into the multiple regression, the only factor giving further significance was VLDL TG in males Probability tables for the occurrence of ST depressions considering age and different levels of LDL cholesterol and VLDL TG were given The importance of simultaneous consideration of both VLDL TG and LDL cholesterol in ST segment depression was evident from the tables Of other "risk factors ' (BP, glucose tolerance, smoking ESR) entered into the regression together with only age or with age and the LPs, only ESR contributed with borderline significance to ST depressions

Various forms of hyperlipidaemia are known to influence the development of ischaemic heart disease (IHD) (22 47) Their high prevalence among IHD patients is also well known (21 24 42) Well defined manifestations of the disease—such as sudden death myocardial infarction and angina pectoris—have been used as criteria of IHD in the accumulation of this knowledge in prospective as well as in retrospective studies Common to these forms of IHD are that there is generally advanced coronary atherosclerosis coronary artery disease (CAD) underlying the IHD (58) although exceptions are known (37 53) However CAD is a process developing over decades Thus for a considerable time the CAD is asymptomatic Little information is available about the presence of silent CAD in apparently healthy subjects with various forms of hyperlipidaemia Although CAD is symptomless for a long time it may give rise to signs of myocardial ischaemia under certain circumstances

One non-invasive technique for the detection of myocardial ischaemia is recording of an ECG during and after exercise Since a demonstration of the presence or absence of significant myocardial ischaemia in various forms of hyperlipidaemia would be of both practical and theoretical interest we have performed exercise ECG on an apparently healthy hyperlipidaemic population We have called this approach to the problem of the association between hyperlipidaemia and IHD *interspective* in contrast to earlier retro- and prospective as well as cross sectional techniques The same approach was used in a parallel study to establish the prevalence of signs of peripheral vascular disease (64)

One of the advantages of this interspective technique compared to the prospective ones is that the interspective sample itself is small It is thus possible to perform elaborate investigations at much smaller effort on an interspective sample We have accordingly determined the serum lipoprotein (LP) concentrations of cholesterol and triglycerides (TG) in this study by ultracentrifugal separation of the three main LP classes the very low (VLDL) the low (LDL) and the high (HDL) density LP Since ie various forms of hyperlipidaemia are caused by ons of one or more of the serum LP classes lesterolaemia may for example be due to an increase of either VLDL LDL or HDL singly or in combination) quantitative LP determinations are essential for analysis and understanding of the relative importance of the various LP classes for the association between IHD and hyperlipidaemia Analyses on the distribution of cholesterol and TG on serum LP are lacking in prospective studies and are still rare in retrospective studies (24 42)

PROCEDURE AND METHODS

Subjects

Metropol Health Control Centre Stockholm examines yearly about 5 000 employees of various companies which offer this to their employees free of charge and during working hours The rate of attendance is 95% The results of the examination are available only to the person himself Subjects who considered themselves healthy and who were not found to have any significant disease by the physicians at Metropol but exhibited marked fasting hyperlipidaemia (cholesterol above 350 mg/100 ml and TG above 3.5 mmol/l) were referred to the Lipid Unit Department of Medicine Karolinska Hospital for further examination From Oct 1970 to March 1974 314 subjects fulfilled the criteria for participation and took part in the study i e about 2% of the basal population Age-matched *controls with serum lipid concentrations below 300* mg/100 ml for cholesterol and 2 0 mmol/l for TG were selected randomly from Metropol

All subjects were seen by one of us (A G O) at the Lipid Unit Case history was taken and clinical examination was performed according to a special questionnaire Smoking was defined as daily smoking of cigarettes pipe or cigars

Based on case history clinical examination and laboratory tests exclusions were made for the following reasons History or presence of myocardial infarction angina pectoris intermittent claudication cerebrovascular disease or other atherosclerotic manifestations hypertension congestive heart failure myocarditis hypothyroidism liver disease gout diabetes anaemia serum electrolyte disturbances and malignant disease Diagnoses were based on the patient s medical record from Metropol and on hospital records or findings at the examination Persons on the following chronic drug treatment were also excluded Hypolipidaemic drugs digitalis diuretics insulin or oral antidiabetics cortisone vasodilators adrenergic β-blocking agents antihypertensives appetite reducing agents anticoagulants thyroid *derivatives*

Altogether 420 subjects with hyperlipidaemia were called to the interspective study Of these 37 failed to appear (not interested 81% moved away and dead 19%) and 69 were excluded for the reasons above The final sample thus consisted of 314 subjects

Two-hundred and twenty two subjects with non-elevated serum lipids were offered the same examinations as the HLP subjects Exclusions were made according to the same criteria as for the HLP group Fifty-one subjects refused 14 did not reply and 4 had moved away Twenty five persons did not fulfil the criteria for participation at the Lipid Unit resulting in a final number of 128 control subjects Reasons for exclusions of hyperlipidaemic subjects and controls are given in detail elsewhere (63)

Chemical methods

Analysis of fasting cholesterol and TG concentrations was repeated twice All cholesterol (14) and TG (50) analyses were performed on frozen serum samples at King Gustaf V Research Institute with semiautomated procedures on an AutoAnalyzer Model I (Technicon Co)

About three months after the hyperlipidaemia had been *diagnosed the type of hyperlipoproteinaemia (HLP)* was classified according to the principle of Fredrickson et al (36) as modified by Beaumont et al (10) but based on quantitative LP analysis Serum LPs were separated in the preparative ultracentrifuge into VLDL LDL and HDL at densities of 1 006 and 1 063 and the cholesterol and TG contents of these three LP classes were determined (70) The top and bottom fraction after centrifugation at d=1 006 as well as whole serum were subjected to LP electrophoresis according to Lees and Hatch (57) The results of the LP analysis are used here only for the classification of HLP and in regression analysis The latter was only applied in cases in which the sum of the LP cholesterol and TG concentrations was 87–113% of the total concentrations The detailed results are presented elsewhere (63)

Table I *Heart rate PQ time and QRS time (mean ±S E M) at rest in males and females above 35 years with different types of HLP and in controls (C)*

HLP classification according to Beaumont et al (10) N=subjects with normal LP type despite hyperlipidaemia at screening

	Men				Women			
Group	*n*	HR (beats/min)	PQ time (csec)	QRS time (csec)	*n*	HR (beats/min)	PQ time (csec)	QRS time (csec)
C	49	68±1	16 8±0 2	8 3± 1	62	74±1	15 4±0 3	7 7± 1
N	30	70±2	15.5±0 3**	8 4± 1	30	74±2	15 6±0 3	7 6± 1
II A	26	71±2	16 3±0 6	8 0± 1	45	76±2	15 4±0 2	7 8± 2
II B	14	73±3	16 2±0 4	8 1± 2	12	77±4	15 2±0 7	7 4± 1
III	9	70±3	16 2±1 1	8 2± 2	6	70±6	16 8±1 5	7 5± 2
IV	81	76±2***	15 8±0 2**	8 2± 1	30	76±2	15 7±0 3	7 7± 2

* ** *** = significant differences against C group at the 5 1 and 0 1 % levels

The i v glucose tolerance test was performed by the routine method of this hospital and the result was expressed as the *k* value i e the percentage of glucose eliminated per minute ESR was determined by the Westergren method

Men and women in the interspective sample were divided into groups according to type of HLP (Table II) In spite of the high cut-off points for the initial (screening) serum lipids a number of subjects had normal LP values at the second analysis These subjects will be called group N The results of the different groups will be presented in the age categories 36–50 above 50 and all ages No difference in mean age existed between the different HLP groups and control groups within each age category In regression analysis also subjects below the age of 35 were taken into consideration as age was used as an independent variable

The exercise test

Procedure The subjects were instructed to refrain from smoking from the evening before the day of investigation Exercise tests were performed in the sitting position on an electrically braked bicycle ergometer (EM 370 Siemens Elema) controlled by a heart rate (HR) controller unit (EMT 252 Siemens Elema) which automatically adjusts the work load of the bicycle so that a certain preselected HR during work can be obtained (35) The subjects exercised for 6 min on consecutive loads beginning with a HR of 90 beats/min and increasing every 6 min by 20 beats/min The exercise was continued until exhaustion or was stopped in a few cases because marked ECG changes were observed The work load and HR were continuously recorded on an ink pen recorder (Servogor 2) and the total work performed was obtained by electrical integration ECG recordings comprising six limb leads and six chest leads (CR) were taken before and after exercise During exercise six chest leads (CH) were recorded continuously

BP was measured with the cuff method using calibrated manometers Before exercise systolic and diastolic pressures were recorded with the patient in the supine position after 10 min rest During exercise systolic BP was measured during the 5th min at each HR level

Coding of the ST segment The ECG at rest and during exercise was interpreted by one of us (L G E) without knowledge of whether the ECG was recorded from a control or hyperlipidaemic subject A magnifier was used for the visual interpretation and the ECG changes were coded according to the Minnesota criteria adapted for CR leads and modified for ECG recorded during and after exercise (70) The ST level was measured 0 06 sec after the junction point The ST segment depressions in the following called ST depressions were divided into two main categories The first was either horizontal or downward sloping or straight and very slowly ascending (slope <0 4 mV/sec) and will in the following text be called horizontal ST depression According to the magnitude of the ST depression it was divided into five subcategories 4 1 with a ST depression ≥0 15 mV 4 2 ST depression 0 1–0 14 mV 4 3 ST depression 0 05–0 09 mV 4 4 no ST J depression as much as 0 05 mV *but* ST segment >0 05 mV and 4 5 no ST J depression but ST segment depression <0 05 mV The second main category was junctional depressions which were referred to either code 4 6 or 4 7 depending on whether the depression was >0 15 mV or 0 05–0 14 mV If there was any doubt about whether a ST depression should be classified as horizontal or junctional the case was coded as a junctional type

For the classification of exercise reaction changes during different HR immediately after and 2 min after exercise were taken into account

Statistical methods

The statistical evaluation was performed in collaboration with the Department of Statistics (head Erik Leander) Linköpings hogskola Linkoping Sweden For comparison of means Student s *t* test unpaired data separate variance estimation was used Degrees of freedom were approximated as the number of observations in the smallest of the two groups minus 1 This is a conservative method in the sense that the usual method for determination of the number of degrees of freedom the Satterthwaite approximation (28) gives a number of degrees

One non invasive technique for the detection of myocardial ischaemia is recording of an ECG dur ing and after exercise Since a demonstration of the presence or absence of significant myocardial ischaemia in various forms of hyperlipidaemia would be of both practical and theoretical interest we have performed exercise ECG on an apparently healthy hyperlipidaemic population We have called this approach to the problem of the association be tween hyperlipidaemia and IHD *interspective* in contrast to earlier retro and prospective as well as cross sectional techniques The same approach was used in a parallel study to establish the prevalence of signs of peripheral vascular disease (64)

One of the advantages of this interspective tech nique compared to the prospective ones is that the interspective sample itself is small It is thus possi ble to perform elaborate investigations at much smaller effort on an interspective sample We have accordingly determined the serum lipoprotein (LP) concentrations of cholesterol and triglycerides (TG) in this study by ultracentrifugal separation of the three main LP classes the very low (VLDL) the low (LDL) and the high (HDL) density LP Since the various forms of hyperlipidaemia are caused by vations of one or more of the serum LP classes ypercholesterolaemia may for example be due to n increase of either VLDL LDL or HDL singly or in combination) quantitative LP determinations are essential for analysis and understanding of the relative importance of the various LP classes for the association between IHD and hyperlipidaemia Analyses on the distribution of cholesterol and TG on serum LP are lacking in prospective studies and are still rare in retrospective studies (24 42)

PROCEDURE AND METHODS

Subjects

Metropol Health Control Centre Stockholm examines yearly about 5 000 employees of various companies which offer this to their employees free of charge and during working hours The rate of attendance is 95% The results of the examination are available only to the person him self Subjects who considered themselves healthy and who were not found to have any significant disease by the physicians at Metropol but exhibited marked fasting hyperlipidaemia (cholesterol above 350 mg/100 ml and TG above 3 5 mmol/l) were referred to the Lipid Unit De partment of Medicine Karolinska Hospital for further examination From Oct 1970 to March 1974 314 subjects fulfilled the criteria for participation and took part in the study i e about 2% of the basal population Age matched controls with serum lipid concentrations below 300 mg/100 ml for cholesterol and 2 0 mmol/l for TG were selected randomly from Metropol

All subjects were seen by one of us (A G O) at the Lipid Unit Case history was taken and clinical examina tion was performed according to a special questionnaire Smoking was defined as daily smoking of cigarettes pipe or cigars

Based on case history clinical examination and labora tory tests exclusions were made for the following rea sons History or presence of myocardial infarction angi na pectoris intermittent claudication cerebrovascular disease or other atherosclerotic manifestations hyper tension congestive heart failure myocarditis hypo-thyroidism liver disease gout diabetes anaemia serum electrolyte disturbances and malignant disease Diagno ses were based on the patient s medical record from Metropol and on hospital records or findings at the examination Persons on the following chronic drug treatment were also excluded Hypolipidaemic drugs di gitalis diuretics insulin or oral antidiabetics cortisone vasodilators adrenergic β blocking agents antihyperten sives appetite reducing agents anticoagulants thyroid derivatives

Altogether 420 subjects with hyperlipidaemia were cal led to the interspective study Of these 37 failed to appear (not interested 81% moved away and dead 19%) and 69 were excluded for the reasons above The final sample thus consisted of 314 subjects

Two-hundred and twenty two subjects with non-ele vated serum lipids were offered the same examinations as the HLP subjects Exclusions were made according to the same criteria as for the HLP group Fifty-one subjects refused 14 did not reply and 4 had moved away Twenty five persons did not fulfil the criteria for participation at the Lipid Unit resulting in a final number of 128 control subjects Reasons for exclusions of hyperlipidaemic sub jects and controls are given in detail elsewhere (63)

Chemical methods

Analysis of fasting cholesterol and TG concentrations was repeated twice All cholesterol (14) and TG (50) analyses were performed on frozen serum samples at King Gustaf V Research Institute with semiautomated procedures on an AutoAnalyzer Model I (Technicon Co)

About three months after the hyperlipidaemia had been diagnosed the type of hyperlipoproteinaemia (HLP) was classified according to the principle of Fredrickson et al (36) as modified by Beaumont et al (10) but based on quantitative LP analysis Serum LPs were separated in the preparative ultracentrifuge into VLDL LDL and HDL at densities of 1 006 and 1 063 and the cholesterol and TG contents of these three LP classes were determined (20) The top and bottom fraction after centrifugation at $d=1\ 006$ as well as whole serum were subjected to LP electrophoresis according to Lees and Hatch (52) The results of the LP analysis are used here only for the classification of HLP and in regression analysis The lat ter was only applied in cases in which the sum of the LP cholesterol and TG concentrations was 87–113% of the total concentrations The detailed results are presented elsewhere (63)

segment			T wave items		
1-4 7			5 1-5 4		
1	36-50	>50	All	36-50	>50
	0	0	16	4	30
	7	0	20	14	25
	0	13	15	18	13
	0	13	29	17	38
	0	0	18	0	33
	2	0	37*	28*	50
	0	5	15	9	18
	0	11	20	18	21
	13	16	29	25	30
	0	13	42	50	38
	0	33	50	33	67
[illegible]	0	19	37	11	48*

of T wave changes Ectopic beats were recorded in only a few cases In addition pathological Q waves were seen only occasionally and most often in the older age groups ST depressions at rest were rare in women almost exclusively in the older age groups and in none of the male controls T wave changes were more prevalent especially in the older age groups They were significantly more frequent in young male and old female type IV groups than in controls

ECG in standing position

T wave changes in the standing position (orthostatic test) (Table III) were in general more frequent than at rest In males but not in females significantly more T wave changes were observed in N and IV HLP groups than in controls This difference was due to higher proportions of 5 4 i e low T waves in the HLP groups

The exercise test

Working capacity The working capacity of subjects in the interspective study is reported elsewhere (35) In summary all male HLP groups had lower working capacity than controls Female type II A had a lower working capacity than controls The possible explanation of these findings is discussed in a preceding paper (35) No differences in mean HR at the end of exercise or in total time exercised were found between the HLP and control groups

Most marked symptoms during exercise The most marked symptoms during exercise were exhaustion fatigue or leg discomfort and were the reason for stopping exercise (%) in the following groups (males/females) Control group 96/97 N group 97/93 II A 100/93 II B 93/92 III 89/100 IV 98/100 Typical angina pectoris (Minnesota criterion 12 1) was present in one type III and in one type IV male The man with type III HLP had ST segment depressions 4 1 and the man with type IV HLP 4 3 according to the Minnesota criteria One male control and one male in the N group complained of typical intermittent claudication The control subject had a normal and the HLP subject had a pathological peripheral circulation as estimated by digital pulse plethysmography (64)

Arrhythmias Ventricular premature beats were found but only in small numbers the frequency varying between 15 and 30 % in the different groups with no significant difference between HLP and control groups in either sex Supraventricular premature beats were rarer and the frequencies did not

Table III *Percentage of subjects above 35 years with T wave changes (5 1–5 4) in standing position in the different groups of HLP and in controls (C)*

	Men			Women		
Group	5 1–5 3	5 4	5 1–5 4	5 1–5 3	5 4	5 1–5 4
C	6	10	16	16	19	36
N	3	33	40	20	23	43
II A	4	12	15	18	31	49
II B	14	14	29	25	8	33
III	11	33	44	0	33	33
IV	17	31	48	17	27	43

Symbols as in Table I

Table IV *Percentage of subjects with ST segment depression according to the Minnesota code during and/or after exercise in groups with different types of HLP and in controls (C)*

	Men								Women							
Group	*n*	4 1	4 2	4 3	4 4	4 5–4 7	4 1–4 3	4 1–4 4	*n*	4 1	4 2	4 3	4 4	4 5–4 7	4 1–4 3	4 1–4 4
Age group 36–50																
C	26	0	0	8	0	8	8	8	22	0	0	9	14	18	9	23
N	14	0	0	29	0	21	29	29	11	9	9	36	9	27	55	64
II A	11	0	27	9	9	0	36	45*	8	0	13	13	0	13	25	25
II B	6	0	0	50	0	33	50	50	4	0	0	50	25	0	50	75
III	3	0	0	0	0	0	0	0	3	0	0	67	0	0	67	67
IV	47	2	2	23	4	32	28	32	9	0	11	0	33	11	11	44
Age group >50																
C	23	4	9	9	4	22	22	26	39	5	8	18	13	18	31	44
N	16	6	19	0	6	25	25	31	19	5	11	26	16	21	42	58
II A	15	13	13	27	0	20	53	53	37	11	16	32	3	16	59*	62
II B	8	25	25	25	0	13	75*	75*	8	25	13	38	0	25	75*	75
III	6	17	17	17	50	0	50	100**	3	0	0	0	0	100	0	0
IV	34	12	3	24	12	26	38	50	21	5	14	29	10	24	48	57
Age group >35																
C	49	2	4	8	2	14	14	16	61	3	5	15	13	18	23	36
N	30	3	10	13	3	23	27	30	30	7	10	30	13	23	47*	60
II A	26	8	19	19	4	12	46**	50**	45	9	16	29	2	16	53**	56
II B	14	14	14	36	0	21	64*	64**	12	17	8	42	8	8	67**	75
III	9	11	11	11	33	0	33	67**	6	0	0	33	0	50	33	33
IV	81	6	2	23	7	30	32*	40	30	3	13	20	17	20	37	53

Symbols as in Table I Tests for significance against controls were calculated only for 4 1–4 3 and 4 1–4 4

between HLP and control groups One 56 year-old man developed a ventricular tachycardia during exercise He had a type II A HLP with an initial cholesterol concentration of 474 mg/100 ml and an LDL cholesterol concentration of 300 mg/100 ml and one woman in the control group developed supraventricular tachycardia during exercise

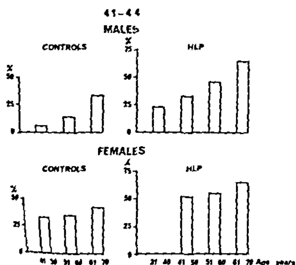


Fig 1 Frequency of ST depressions (Minnesota code 4 1–4 4) in subjects with hyperlipoproteinaemia (*HLP*) and in controls in different age groups

ST depressions during and after exercise

The frequency of ST depressions of different magnitude during and after exercise is given in Table IV and Figs 1–3

ST depressions by age and sex The frequency of ST depressions increased with age in both HLP and control subjects (Fig 1) It was also higher in females than in males regardless of lipid levels The more marked ST depressions 4 1–4 2 were rare in both males and females before the age of 50 After that age the frequency was higher and of the same magnitude in both sexes

HLP and ST depressions (Table IV and Figs 2 and 3) The frequency of ST depressions was higher in all types of HLP than in the control groups In younger subjects the prevalence of ST depressions was significantly higher in type II A and type IV males and group N females In older age groups significantly higher frequencies were seen in males with types II B and III and in females with types II A and II B HLP When all ages were taken together

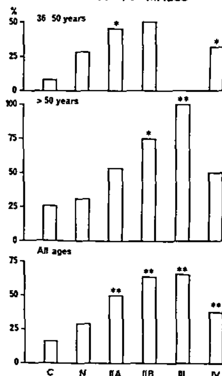


Fig 2 Frequency of ST depressions (Minnesota code 4 1-4 4) in males with different types of hyper lipoproteinaemia and in controls (C) * ** = significant difference against controls at the 5 and 1% levels respectively

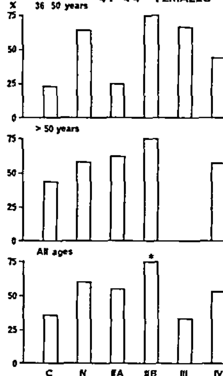


Fig 3 Frequency of ST depressions in females with different types of hyperlipoproteinaemia and in controls * = significant difference against controls at the 5% level

significantly higher frequencies were found in all male HLP groups except N and female N II A and II B (Table IV) The frequency of ST depressions 4 1-4 2 reflected approximately the prevalence of 4 1-4 4 i e it was most abundant in types II A and II B HLP However the frequencies were too small to show significant differences

Blood pressure and ST depressions All groups were subdivided into two one having lower and one higher values for diastolic BP (cut-off point 85 mmHg) (Table V) and i v glucose tolerance (cut-off point $K=1\ 10$) (Table VI) All groups were also divided into non smokers smokers and ex smokers (Table VII) There was no significant difference in

Table V *Total number and percentage of subjects with ST segment depressions (4 1-4 4) in the different HLP groups and in controls (C) divided according to diastolic blood pressure*

	Men				Women			
	<85 mmHg		≥85 mmHg		<85 mmHg		≥85 mmHg	
Group	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
C	25	20	24	13	45	40	14	29
N	19	21	11	45	20	50	10	80
II A	15	40	11	64**	30	57	15	53
II B	8	63	6	67	5	80	7	71
III	2	50	7	71	5	40	1	0
IV	35	40	45	40*	16	50	12	67
All HLP	79	38	80	49	76	54	45	64*

Symbols as in Table I There were no significant differences between low and high diastolic BP

Table VI *Number and percentage of subjects with ST segment depressions (4 1–4 4) in different HLP groups divided according to i v glucose tolerance (K value)*

Group	Men				Women			
	K≥1 10		K<1 10		K≥1 10		K<1 10	
	n	%	*n*	%	*n*	%	*n*	%
N	20	30	10	30	27	59	1	100
II A	18	39	8	75	36	50	9	78
II B	9	67	5	60	7	71	3	67
III	4	50	5	80	5	40	1	0
IV	42	36	38	45	20	50	10	60
All HLP	93	39	66	50	95	54	24	67

Symbols as in Table I There were no significant differences between subjects with high and low glucose tolerance

the frequency of ST depressions between subjects with high and low BP in any group This was also true when all HLP subjects were pooled into one group, when 90 mmHg was used as cut off point instead of 85 and when systolic BP with cut off point 140 mmHg was used instead of diastolic BP

It is of interest however that when the frequency of ST depressions in the HLP groups was compared with the frequency in the control group a significantly higher frequency was seen only in the high pressure subjects of the HLP groups This was particularly true for males

Glucose tolerance and ST depressions The division of the subjects according to the results of the i v glucose tolerance test did not reveal any significant differences between high and low responders in any group

Smoking and ST depressions Non smokers had a higher frequency of ST depressions than smokers in 12 of the 14 groups Statistical significance existed only in two groups however male type II A and female controls When comparing the frequencies of ST depressions between the HLP groups and the controls significantly higher values for HLP groups were seen both among smoking and non smoking men For the women a higher frequency was encountered only among smokers

Regression analysis on the dependence of ST depressions on LP concentrations It is evident from the results in Table IV and Fig 1 that the frequency of ST depressions during exercise increased with age and with the various types of HLP Since as discussed elsewhere (63) the concentrations of VLDL and LDL vary continuously and thus the various types of HLP are arbitrarily defined we have studied further the relations between HLP and ECG changes by regression analysis considering not types but separate LP concentrations (Tables VIII and IX) A number of different models for de-

Table VII *Number and percentage of subjects with ST segment depressions (4 1–4 4) in the different HLP groups and in controls (C) divided into non smokers smokers and ex smokers*

Group	Men							Women						
	Non smokers			Smokers		Ex smokers		Non smokers			Smokers		Ex smokers	
	(*n*)	(%)	*p*ᵃ	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	*p*ᵃ	(*n*)	(%)	(*n*)	(%)
C	21	24	>0 05	24	13	4	0	45	44	<0 05	13	8	3	33
N	11	27	>0 05	13	23	5	60	17	65	>0 05	11	64 **	2	0
II A	11	91***	<0 01	13	23	2	0	31	61	>0 05	13	38	1	0
II B	1	100	>0 05	12	67*	1	0	7	71	>0 05	5	80	0	0
III	3	67	>0 05	5	60	1	100	5	40	>0 05	1	0	0	0
IV	30	43	>0 05	46	37	5	40	8	50	>0 05	19	58**	3	33
All HLP	56	52*	>0 05	89	36*	14	64	68	60	>0 05	49	55**	5	20

Symbols as in Table I ᵃ Significance of the difference between non smokers and smokers

Table VIII *Linear regression analysis of ST segment depressions (4 1–4 4) on age plus serum lipids lipoproteins diastolic BP smoking glucose tolerance ESR W_{150} and weight/height (W/H) index in men and women with types II A II B III and IV HLP and in controls with non elevated serum lipids*

b=regression coefficient t=t value for significance of b (b/s_b) R=correlation coefficient

	Men (n=166)					Women (n=135)				
	For age		For parameter		For both	For age		For parameter		For both
Parameter	b	t	b	t	R^2	b	t	b	t	R^2
Age (y)	0 016	4 49	–	–	0 109	0 014	2 90	–	–	0 059
Total cholesterol (mg/100 ml)	0 015	4 58	0 0022	4 03	0 190	0 010	2 15	0 0011	1 63	0 078
log total TG (mmol/l)	0 015	4 44	0 0097	1 98	0 130	0 012	2 62	0 17	2 60	0 105
[a]log VLDL cholesterol (mg/100 ml)	0 015	4 43	0 076	1 82	0 127	0 012	2 59	0 13	2 18	0 092
log VLDL TG (mmol/l)	0 015	4 46	0 066	1 88	0 128	0 013	2 79	0 12	2 37	0 097
LDL cholesterol (mg/100 ml)	0 016	4 65	0 0019	3 55	0 173	0 011	2 27	0 0011	1 61	0 078
LDL TG (mmol/l)	0 015	4 23	0 45	2 65	0 146	0 012	2 58	0 38	2 44	0 100
HDL cholesterol (mg/100 ml)	0 016	4 56	−0 0038	−1 47	0 121	0 014	2 96	−0 0018	−0 68	0 063
HDL TG (mmol/l)	0 016	4 48	0 094	0 29	0 110	0 013	2 65	0 58	1 48	0 075
Diastolic BP (mmHg)	0 015	4 30	0 0018	0 46	0 111	0 014	2 80	−0 004	−0 91	0 059
Smoking no–yes (0–1)	0 016	4 50	−0 080	−1 13	0 116	0 013	2 73	0 044	−0 50	0 061
ESR (mm/h)	0 014	3 82	0 0045	1 75	0 128	0 011	2 51	0 0070	2 29	0 093
W_{150}[a] (kpm/min)	0 015	4 38	−0 0002	−1 50	0 125	0 012	2 44	−0 0002	−0 71	0 053
Glucose tolerance k[b]	0 017	4 16	−0 109	−1 53	0 151	0 013	1 57	−0 020	−0 20	0 031
W/H index	0 015	4 38	0 29	0 88	0 114	0 013	2 73	0 57	1 75	0 078

[a] *Working capacity at a HR of 150/min reached in 164 males and 121 females*
[b] The equation includes only subjects with HLP (133 men and 81 women)
[c] Calculated as body weight kg/body height cm−100

scription of the relations were tried The highest correlation coefficients were achieved if the N groups were excluded As it is conceivable that a number of subjects in the N groups had by their own actions decreased their serum LP concentrations below their habitual levels it was considered justifiable to exclude them As ST depressions 4 1–4 4 gave higher correlation coefficients than 4 1–4 3 only the results with the former criterion are reported Furthermore the logarithms of VLDL cholesterol and TG were more efficient than the ordinary values in describing the relation to ST depressions

The results of regression analysis on the dependence of ST depressions on age together with one serum lipid or one LP as a *linear* term are given in Table VIII Age gave invariably the most significant correlation to ST depressions and was therefore always used as one term in the regression analysis For men total serum cholesterol was highly correlated to ST depressions while total TG were less but significantly correlated For women total TG were positively related while cholesterol was not Using single LPs as linear terms LDL cholesterol gave the strongest correlation for males followed by LDL TG For females LDL TG gave the strongest correlation followed by VLDL TG while LDL cholesterol did not come out significantly HDL cholesterol was negatively but insignificantly related to ST depressions in both males and females

Higher correlation coefficients were in general reached if a *quadratic* term was used in addition to the *linear* (Table IX) With this method total serum cholesterol was significantly correlated to ST depressions for both sexes although most for men Total TG were also but less significantly correlated to ST depressions LDL cholesterol had the highest correlation coefficient now for both men and women when used as a single LP in addition to

Table X *Probability (p) of ST segment depressions (4 1–4 4) with given concentrations of VLDL TG and LDL cholesterol in 50 year old males (y) according to the equation p=−1 16+0 014 y+0 084 ᵉlog VLDL TG+0 0071 LDL cholesterol−0 000013 (LDL cholesterol)²*

LDL cholesterol (mg/100 ml)									
340	35	41							
310	38	44	50						
280	39	45	51	57	60				
250	38	44	50	56	59	61			
220	35	41	46	52	56	58			
190	29	35	41	46	50	52	58		
160	21	27	33	38	42	44	50		
130	11	17	22	28	32	34	40	46	
100	− 02	04	10	16	19	21	27	33	39
70	− 16	− 11	− 05	01	04	07	12	18	24
	0 2	0 4	0 8	1 6	2 4	3 2	6 4	12 8	25 6
VLDL TG (mmol/l)									

X and XI As the best regression equation was obtained with negative quadratic terms for LDL the influence of LDL cholesterol was particularly portant for the lower range of LDL cholesterol here at all VLDL levels doubling to trebling of the probability for the presence of ST depressions occurred when LDL increased from 100 to 200 mg/100 ml Above a LDL cholesterol level of 220 mg/100 ml there was little or no further increase in the probability of ST depression

For VLDL the picture is somewhat different as the regression component was linear which implies that the probability of ST depression will always increase with increasing VLDL TG However since the best fit was obtained with logarithmic values here too it was particularly the increase at lower levels which was most important When the VLDL TG increased from 0 2 to 1 6 mmol/l the probability of ST depressions was doubled to trebled within the normal LDL range

DISCUSSION

The significance of ST depressions during exercise

The resting ECG is an insensitive instrument in detecting subclinical CAD It could be normal even in cases with advanced sclerotic changes in the coronary arteries (16) However in several studies in men an ECG recorded during or after exercise has been shown to be a rather sensitive test method and to have a rather high predictive value (specificity) in detecting silent CAD (1 2 5 9 12 19 27) It is generally although not universally accepted that certain transient abnormalities in ventricular repolarization (ST depression) appearing during or after exercise are manifestations of reversible myocardial ischaemia Thus the horizontal ST depressions (Minnesota code 4 1–4 3) in connection with exercise are generally called ischaemic due to the high correlation to the incidence of IHD (31 34 39 41 49) Ischaemic ST depression is however not specific for CAD and could be seen in other heart disorders such as a cardiomyopathy, status post myocarditis or as an expression for increased sympathetic tone Furthermore ST depressions can be induced by anaemia electrolyte disturbances or treatment with cardiotropic drugs In the absence of these conditions this ECG abnormality can be considered as an indicator of myocardial ischaemia often due to CAD In the present study subjects with these conditions were excluded as far as possible

The specificity of horizontal ST depressions to predict IHD has been reported to vary between 60 and 85% and increases if one only accepts ST depressions of a magnitude of 0 1 mV (Minnesota code 4 1–4 2) or more (38 59 67) However in that case the sensitivity decreases especially if the work load is far from maximal for example Master s

Table XI *Probability (p) of ST segment depressions (4 1–4 4) with given concentrations of VLDL TG and LDL cholesterol in 50 year old females (y) according to the equation p=−0 84+0 0088 y+0 071 ᵉlog VLDL TG+0 0080 LDL cholesterol−0 000016 (LDL cholesterol)²*

LDL cholesterol (mg/100 ml)									
340	28	33							
310	36	41	46						
280	41	46	51	56	59				
250	44	49	54	58	61	63			
220	43	48	53	58	61	63			
190	40	45	49	54	57	59	64		
160	33	38	43	48	51	53	58		
130	24	29	34	38	41	43	48	53	
100	11	16	21	26	29	31	36	41	46
70	− 04	01	06	11	14	16	21	26	30
	0 2	0 4	0 8	1 6	2 4	3 2	6 4	12 8	25 6
VLDL TG (mmol/l)									

double step test (67) One way to increase the sensitivity is to use a work load near maximum (17 49) The knowledge of this type of exercise testing in detecting silent CAD is not yet as extensive as from the earlier submaximal tests but several reports (16 29 30 39 49) indicate a high sensitivity and a good specificity If one includes horizontal ST depressions of the magnitude of 0 05 mV (Minnesota code 4 3–4 4) the sensitivity increases but there is a risk of a lower degree of specificity

The junctional ST depressions (Minnesota code 4 6–4 7) are mostly thought to be functional changes without prognostic significance (3 13 16) but there are both experimental studies (26) and epidemiological observations (32) which indicate that they could be early signs of ischaemia They have however not been included in our analysis of the role of HLP for ECG changes

Horizontal ST depression during exercise test in individual subjects does not definitely indicate that an individual has a grave prognosis Several reports have shown that an abnormal responder has a substantially higher risk of developing IHD than normal responders (38 67) Only one study is available in which coronary angiography has been performed in *asymptomatic* men with ST depressions during near maximal HR (40) The frequency of angiographically demonstrated CAD was 53% This should be compared to 5–7% in normal populations (40) Higher frequencies have been reported in subjects with overt CAD such as angina pectoris (8 54 56 68) Nevertheless it could be argued that the abnormal ECG reaction during exercise has a poor specificity for the diagnosis of CAD shown at angiography However these two techniques of detecting CAD measure different things Exercise ECG reaction gives information on the metabolic function at the cellular level while the arteriography only demonstrates the morphological changes in the coronary arteries Therefore complete conformity of the two methods cannot be expected

In healthy subjects the frequency of horizontal ST depressions during exercise varies with sex and age (4 16 73 77) At the age of 40 the frequency in males is about 10% and in females about 20% The corresponding frequencies at the age of 55–60 are 35 and 50% respectively (4)

While myocardial infarction is more common in males than in females the prevalence of angina pectoris is about the same in the two sexes (11) or even higher in females (4) There are however virtually no data available on the sensitivity and specificity of exercise ST depressions for the prediction of myocardial infarction or angina pectoris in females The exercise electrocardiographic response in female subjects is recently reported to be similar to the male with regard to sensitivity and specificity as evaluated by coronary angiography (54B)

The frequency of ST depressions in the control groups of the present study are in agreement with previous figures as regards sex and age in healthy subjects (4 18 77)

Single lipoproteins and ST depressions

Since all types of HLP at least for the men had increased frequency of ST depressions it would at first sight seem reasonable to ascribe this to the particular LP abnormality being diagnostic for each type Thus for the group with *type II A* HLP the raised LDL cholesterol (or some associated abnormality) would appear to be the most likely factor responsible for the ST depression Correspondingly for the other groups *type II B* LDL cholesterol+VLDL TG *type III* β VLDL *type IV* VLDL TG The situation is however much more complex because LP abnormalities other than the diagnostic ones are present in the various types of HLP (63) For instance compared to the control group HDL cholesterol was low in types II B III and IV HLP Furthermore HDL cholesterol has been reported to be low in survivors of myocardial infarction (21 24 61) and is lower in males than in females (23 69) Against this background the hypothesis was recently put forward that a reduction in serum HDL may accelerate the development of atherosclerosis possibly by an impairment of the normal clearance of cholesterol from the arterial wall (57) Another non diagnostic LP abnormality in HLP is the increased concentration of LDL TG occurring with or without concomitant increase in LDL cholesterol It has been found recently that the hypertriglyceridaemia present in survivors of myocardial infarction was in about 25% due to elevation of only LDL TG (24)

In an attempt to obtain information about the possible pathogenetic importance of the various LP classes in the development of ST depression we have used regression analysis with ST depressions as the dependent variable This was done in order

to evaluate if there was a continuous quantitative relation between the concentrations of any LP class(es) and the occurrence of ST depressions Such a quantitative relation would be compatible with although it would not establish a direct causa tive relationship between the LP class and the ST depression In all these regressions age was also introduced because of the strong age dependence of the occurrence of ST depression

For *LDL cholesterol* the linear regression analysis showed a positive relation for men but not for women The association was improved by quadratic regression and then became significant also for females Applying the quadratic regression equation ST depressions increased continuously with increasing LDL concentrations until LDL cholesterol reached a value of 268 and 235 mg/100 ml for men and women respectively (Values obtained by derivation of the regression equation Table IX)

For *VLDL TG* the ST depression showed a sig nificant linear relation for women but not for men Upon quadratic regression analysis ST depres were significantly dependent on VLDL TG) for males and the relation for females was ly improved Thus ST depressions increased ith increasing VLDL TG until a value of 2 1 and 3 2 mmol/l was reached for males and females re spectively (Values obtained by derivation of the regression equation Table IX)

LDL TG showed a positive relation with ST de pressions which was slightly stronger with the quadratic than with the linear regression In all in stances LDL TG gave a slightly higher correlation to ST depressions than did VLDL TG

HDL cholesterol showed a negative relation to ST depressions Its contribution to the total R^2 for regressions was however smaller than that of the other LP classes

The interpretation of these regressions for the dependence of ST depressions on age and one LP variable at a time is difficult because strong interre lations exist between the LP variables The fact that the equations with the best fit predict that the probability of ST depressions first increased with increasing concentrations of LDL cholesterol as well as VLDL TG but at a certain level started to decrease may in part be due to the strong negative relationship between these LP values In other words if both LPs particularly at moderate levels were of considerable importance for the appearance of ST depressions as suggested by the quadratic regression equation the influence of one of them would be cancelled out at higher levels by the fact that the concentrations of the other one would then be low The regression equation for the relation between LDL cholesterol and VLDL TG ($r = -0\ 77$) in this material (63) indicates that when VLDL TG becomes 2 1 mmol/l the value at which the equa tion for the dependence of ST depressions on VLDL TG predicts that the probability of ST de pression would start to decrease LDL cholesterol would be around 180 mg/100 ml Thus when VLDL TG continued to increase from 2 1 mmol/l the LDL cholesterol value would fall from 180 mg/100 ml and apparently the latter phenomena would be more beneficial than the detrimental effect of the former Correspondingly when LDL cholesterol becomes 268 mg/100 ml VLDL TG would be 0 64 mmol/l and the same reasoning as above could be applied

Combined LPs and ST depression It is thus con ceivable that when only one LP class is used in regression analysis the probability of ST depres sion will first increase with increasing LP concen tration but later start to decrease due to the hidden beneficial effect of the concomitant fall in other LP For that reason *both* VLDL TG and LDL cholesterol were entered into the regression analysis with age included This resulted in a significantly higher R^2 value in males than when only one LP class was used indicating that ST de pressions depend on both these LPs simultaneous ly In females introduction of VLDL TG into the equation did not result in a significantly higher R^2 but gave the highest R^2 of singly added LPs (iden tical for HDL cholesterol) The regression equation *changed when both VLDL TG and LDL choles* terol were considered simultaneously The ST de pression now showed the best dependence (highest R^2) when VLDL TG contained only a linear and positive component For LDL cholesterol the rela tion still contained the quadratic component but the concentration in which the equation no longer predicts any increase with rising LDL cholesterol was higher around 280 mg/100 ml

HDL cholesterol did not show any significant relation to ST depressions particularly not when VLDL TG were already introduced into the regres sion This does not rule out that HDL cholesterol might be of importance in this regard in subjects with less marked HLP and perhaps particularly in normolipidaemic subjects However in a recent

study on survivors of myocardial infarction it was found that the low HDL cholesterol of these patients was entirely due to the hypertriglyceridaemia (24) This is in agreement with the present results where HDL cholesterol had no influence when VLDL TG had been considered

Because of these relations between ST depressions and LPs it seems reasonable to ascribe the ST depression to an effect of elevation of VLDL and LDL or some associated abnormality being either caused by or the cause of the HLP

Several lines of evidence suggest that VLDL and LDL may be taken up directly by the arterial wall as suggested in the LP filtration hypothesis of atherosclerosis (65) LDL (74) and VLDL (79) are claimed to be present in the arterial wall Furthermore Smith and Slater (75) found that the amounts of LDL recovered at autopsy in aortic intimas were positively and highly significantly correlated to the serum cholesterol levels some time before death Also the glucoseaminoglucans of aortic wall interact with and bind LDL and VLDL (45) Considering these earlier studies it seems possible that our finding of increased frequency of ST depressions with increasing VLDL and LDL concentrations may be the result of an increased atherosclerosis due to raised LP concentrations in the coronary arteries

In a recent report Brensike et al (15) estimated the frequency of ST depression during exercise at near maximal HR in 545 21–55 year-old asymptomatic subjects with LDL cholesterol within the upper 5% As neither total TG nor VLDL TG were measured it is not known to what extent this material comprised types II A and II B HLP ST depressions were found in 12 and 6% of males and females respectively It was concluded that asymptomatic patients with type II HLP have 2–5 times increased frequency of ST depression Although the frequencies were substantially higher in the present study the relation between HLP and control subjects was of similar magnitude The reason for the observed differences between the two studies might partly be due to higher cut off points for initial cholesterol and TG higher age and HR closer to maximal HR in the present study Results similar to those of Brensike et al were obtained by Motta et al (60) with regard to males while no increased frequency of ST depressions was seen in female HLP subjects The quantitative role of LDL or other LPs was not assessed in these studies

Total serum cholesterol and TG versus LPs in relation to ST depressions

In most epidemiological studies relating increased serum lipid concentrations to atherosclerosis only *total* concentrations of cholesterol and TG have been used However total serum lipid concentrations are the sum of VLDL LDL and HDL lipids and these different LPs have different biochemical metabolic and pathogenetic properties While increased concentrations of VLDL and LDL have been associated with IHD HDL cholesterol has been reported to be low in survivors of myocardial infarction (24) It has been suggested that a reduction of serum HDL may accelerate the development of atherosclerosis (57)

Total cholesterol is thus the sum of on the one hand LP fractions potentially *deleterious* to the arterial wall when increased (VLDL and LDL cholesterol) and on the other a LP fraction which might be potentially *beneficial* to the arterial wall when increased (HDL cholesterol) It was therefore of interest to compare the R^2 of total cholesterol and TG with R^2 of cholesterol and TG values in the different LP fractions added to each other Regression analysis was therefore performed on the dependence of ST depressions on total serum cholesterol and TG and also on VLDL + LDL + HDL cholesterol In males R^2 for total serum cholesterol was 0 21 For only LDL cholesterol R^2 was 0 20 but the value when all different LPs were taken together was 0 23 In females the corresponding R^2 were 0 12 0 13 and 0 14

R^2 for total serum TG was 0 16 in males For only VLDL TG it was 0 15 for only LDL TG 0 16 and for all LP TGs 0 18 The corresponding figures for females were 0 11 0 10 0 11 and 0 12 Thus the combination of the individual LP lipids invariably gave a higher R^2 than for the total lipid That the difference between the R^2 for total and the combination was not greater may partly be due to the fact that we are here dealing with very excessive HLPs in which the total lipid value is very much dependent on only one LP class because of its excessive concentration In this situation variations in other LP classes such as a decrease in HDL cholesterol have a smaller influence than they might have had in a general population

When discussing the effect of combination of LP classes in the regression analysis it might be appropriate to apply metabolic aspects From that

point of view one would like to consider VLDL TG and LDL cholesterol since the former transports TG (from liver to peripheral tissues) and the latter transports cholesterol (remnant from VLDL catabolism) to catabolism In fact when these two LPs were combined in the regression analysis the highest R^2 was obtained and each LP class had a statistically significant effect for males on ST depression

ST depressions in the different sexes

The frequency of ST depressions in relation to LP concentration showed the same pattern in both sexes although frequencies of ST depressions were generally higher and the significances generally lower in females This suggests that pathogenetic mechanisms as to the LPs in the development of CAD are similar in both sexes but that other mechanisms might explain the difference in the clinical picture and mortality in IHD This is in agreement with a recent study (54B) in which ST depressions during exercise were related to findings on coro angiography showing similar sensitivity and ificity as in males

, arallels could be drawn here to the interesting udy by Oliver (62) of IHD in young women He found serum cholesterol concentrations above 350 mg/100 ml in only 14% of his patients hypertriglyceridaemia was even less common HLP was thus not an important factor for IHD in young female survivors

The influence of diastolic blood pressure on ST depressions in HLP

In hypertension there is a higher frequency of ST depressions not due to ischaemia (44) No subject with hypertension was included in this study However no innocuous limits for BP in the development of IHD exist (48) No difference was seen in the frequency of ST depressions between subjects with low and high diastolic pressure However high BP appeared to have an influence in another way i e the increased frequency of ST depressions in HLP subjects was significant only in the high but not in the low pressure group This difference may at least partly be due to the fact that the high pressure control groups had a lower frequency of ST depression than the low pressure group That diastolic pressure had only little effect on ST depression in these normotensive subjects is supported by the regression analysis Here diastolic BP did not come out as an independent determinant for ST depression either together with age alone (Table VIII) or in combination with LPs (Figs 4 and 5)

The influence of smoking on ST depression

In the present study ST depressions during exercise were less frequent among smokers than among non smokers Before discussing the influence of smoking on ST depressions the following distinctions should be made

Firstly the establishment of smoking as one of the major risk factors for *myocardial infarction* is firm and indisputable (22 46 76 80) The cigarette habit is most strikingly related to the serious lethal manifestation of IHD (46)

Secondly the pathogenetic influence of smoking in the development of *angina pectoris* is less clearcut Although autopsy findings have shown a correlation between the degree of atherosclerosis and the amount of cigarette smoking in man (7) the association of smoking and angina pectoris is dubious (11 33 46 62 71 78)

Thirdly a distinction has to be made between examinations of exercise ECG in subjects stating habitual smoking and studies on the acute effect of smoking on ECG and cardiovascular haemodynamics (6)

The finding of a higher frequency of ST depression among non smokers than smokers has been made before (11 43 51 55) Lundman in his study on smoking in relation to IHD in twins (55) found that it was always the less exposed co-twin who displayed the ST changes during exercise This was considered at least partly to be explained by the fact that the more exposed co twin had discontinued the exercise at a slightly lower HR It was however concluded that smoking is not a significant factor in the causation of coronary heart disease (55) In the present study no essential difference was noted in final HR or maximal work load between smokers and non smokers Thus there must be other explanations for the differences observed in our study

In Bengtsson s study of IHD in women (11) smokers tended to be under represented in the group with coronary ECG This was interpreted as a possible indication that the coronary ECG in women is not mainly a manifestation of IHD However as this finding is also observed in males it

could not invalidate only female coronary ECG (54B)

The reason for the observed difference in the frequency of ST depressions in smokers and non smokers is not known Except for males with type II A HLP the ratio of frequency of ST depressions between non smokers and smokers was highest in the control groups in both men and women This suggests that HLP tends to cancel out the relatively low incidence of ST depression seen in smoking controls In women significant differences were thus found only between smoking controls and smokers in different HLP groups but not between non smoking controls and HLP groups

The influence of glucose tolerance on ST depressions

A decreased glucose tolerance is a frequent finding in ischaemic vascular disease and is reported to be largely independent of serum lipid concentrations in males (25) Glucose tolerance did not influence the occurrence of ST depressions significantly in HLP subjects in this study either when comparing subjects with low and high glucose tolerance or in regression analysis However as glucose tolerance test was not performed on control subjects the results are not fully comparable with the effect of e g LPs and age on the ST depressions In the study of peripheral circulation in asymptomatic HLP (64) subclinical signs of peripheral artery disease were on the other hand often associated with a decreased glucose tolerance

The low power of risk factors to predict ST depressions

It is evident that even when all the following risk factors age LPs blood pressure smoking glucose tolerance and ESR were taken together they could not solely explain the ST depressions as the highest R^2 value in the combined multiple regression analysis was only around 0 25 (R=0 50) This indicates that 25% of the ST depressions can be explained by the factors taken into account This weak relationship may partly be due to the fact that the construction of the multiple regression equation is not ideal However regression analysis with logistic functions did not improve the R^2 value Partly however some of the ST depressions may be due to causes other than ischaemia based upon atherosclerosis of the coronary arteries Then of course risk factors for IHD would not play a role for such ST depressions Another possibility is the presence of risk factors other than those mentioned above Considering that at least in men ST depressions carry an increased risk of myocardial infarction it is important to find out which other factors than age and LP concentrations might be of importance However since this material was selected entirely because of HLP it would seem reasonable to look for only such new risk factors other than LP concentrations of cholesterol and TG within the field of lipid metabolism

In this material of HLP age LDL cholesterol and VLDL TG were the important determinants of ST segment depressions Within the range of LP concentrations present Table X shows that in a *50-year-old man the highest statistical probability of* having ST segment depression was p=0 61 reached with the extreme type II B pattern with LDL cholesterol of 250 mg/100 ml and VLDL TG of 3 2 mmol/l The malignancy of the type II B pattern for ST depressions in both males and females is evident from Tables X and XI and has also been demonstrated in the Stockholm Prospective Study for myocardial infarction in men (22)

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Progression of Coronary Artery Disease in Patients with Stable Angina Pectoris

Arteriographic Ventriculographic and other Correlation Studies

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ABSTRACT **Left ventriculography and selective coronary angiography with determination of resting left ventricular end-diastolic pressures and ejection fraction have been carried out twice on 25 patients with stable angina pectoris The interval between the two angiographic studies varied between 7 and 16 months (mean 12 3) Progression of obstruction amounting to occlusion was found in 4 of 73 arteries studied (5 %) No new lesions occurred Resting end diastolic pressures before and after ventriculography did not change during the observation period Values for ejection fraction were also unchanged, but there was a tendency for them to decrease in patients in whom this parameter had been abnormal at the first catheterization**

By performing coronary cineangiography together with left ventriculography in one and the same patient at different times it is possible to determine the progression rate of atherosclerotic heart disease in living man Such studies have been carried out in order to evaluate structural changes after saphenous vein coronary bypass graft surgery (9 10) but few patients have been reexamined without surgical intervention in between Clearly more information is needed to illustrate the evolution and natural course of coronary artery disease in medically treated patients

The present report documents the changes which occurred in the coronary arteries of 25 patients who had a second angiography on an average of 12 3 months after the first with no surgical intervention in between Only subjects suffering from invalidating but stable angina pectoris with no clinical or ECG evidence of progression of disease in the interval between the first and second exam[illegible] were included In addition to the angi[illegible]graph[illegible] ings the present study includes a compar[illegible] tween data for left ventricular end-dia[illegible] sure and ejection fraction ob[illegible]ained studies and the incidence of abnorma[illegible] left ventricular wall motion

MATERIAL AND METHODS

The study is based on the findings in 25 [illegible] patients (22 men and 3 women) with atheroscler[illegible] nary artery disease referred to us for diagnostic eva[illegible] of their coronary vessels and left ventricular functi[illegible] fore aorto-coronary vein bypass surgery For vari[illegible] reasons the operation was postponed for more than [illegible] months after the initial examination necessitating a new preoperative angiographic study The interval between the two angiographic examinations varied between 7 and 16 months (mean 12.3)

In the interim the patients were treated medically with nitroglycerin and β-blocking agents All had continued but essentially unchanged symptoms of angina pectoris Eight patients were in grade 2 and 17 in grade 3 according to the NYHA classification ECG was normal in 9 patients and showed non-specific ST T wave changes in 12 while 4 patients demonstrated pathological QRS complex indicating old myocardial infarction There was no difference between ECG taken at the first and second examination An abnormal serum lipid pattern (cholesterol >300 mg/100 ml triglyceride >150 mg/100 ml) was found in 20 patients

Selective cinecoronary angiography was carried out according to the technique described by Judkins (13) The coronary arteriograms were performed in the left anterior and right anterior oblique and additional projections when needed The three main coronary arteries—the anterior descending branch and the circumflex branch of the left coronary artery and the right coronary artery with branches—were identified and evaluated separately Each

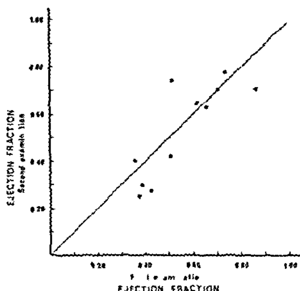


Fig 3 Ejection fraction Values from first and second examination plotted against each other The solid line represents the line of identity

patients (Fig 3) At the first examination preangiographic pressures varied between 4 and 26 mmHg with a mean of 15 1±S D 5 55 At the sec › they varied between 3 and 25 mmHg with a of 15 9±S D 7 67 The small difference be ·en the two means was statistically insignificant (t=0 824 p>0 05)

With a few exceptions the postangiographic left ventricular end-diastolic pressures were higher than those recorded before angiography The rise was of approximately the same magnitude in both studies

Concerning cardiac functions we were unable to demonstrate any difference between the 21 patients with non progressive disease and the small group of 4 patients who were characterized by progressive coronary artery disease

DISCUSSION

Ideally information regarding progression of coronary artery disease should be based on repeat examinations of normals or asymptomatic patients This is not possible Since studies for several reasons must be based on symptomatic patients any conclusions regarding the progression of disease will be biased by selection of patients Firstly such studies will include only survivors of the disease and secondly they usually concern patients who do not improve or even become worse in the interim between the two catheterizations Comparison of coronary arteriograms of patients before and after coronary bypass surgery is not ideal for drawing conclusions regarding the natural course of coronary artery disease as the surgical intervention and grafting may also initiate changes in the ungrafted arteries For several reasons medically treated patients with coronary artery disease with persistent but stable symptomatology would seem to be suitable for comparison of repeated coronary arteriograms in the study of the disease s natural course

The patients reported here were treated medically and coronary arteriography was repeated because of persistent but not progressive symptomatology and reconsideration of surgical treatment In these patients we found a relatively low rate of progression of the obstructive coronary artery disease and most arteries were found to be unchanged at the second examination approximately 12 months after the first Arteries which were perfectly normal at the inception of the study did not develop evidence of coronary artery disease on subsequent arteriograms despite the fact that the average age of the patients was nearly 50 years Interestingly Gensini and Kelly (8) found that 19 of 20 patients with normal coronary arteries at the initial examination maintained this normality on subsequent arteriograms when followed for a period of months to years According to these authors if an individual has reached middle age with angiographically normal vessels he has an excellent chance of maintaining a normal coronary system throughout his life Whether this is true only provided that all three main coronary arteries are open or valid even when only one or two vessels are open is not known Maurer et al (16) found in studies of patients with and without coronary vein bypass that new occlusions occurred in 2% of previously normal segments of an ungrafted artery New stenosis not amounting to total occlusion was evident in a further 10% of the ungrafted arteries free of disease (mean interval 11 7 months)

In the present study progression of disease was limited to the vessels in which preexisting coronary obstructions were found at the first examination And even in vessels with extensive disease the rate of progression appeared to be relatively slow and was demonstrable in 4 (6%) of 66 arteries with preexisting disease The progression rate found by us is the same or lower than most of the results

reported in studies of non grafted arteries in follow up after bypass surgery with the same interval between the two arteriograms Malinow et al (14) reported that occlusions occurred in 6% of ungrafted arteries examined after an interval of approximately 8 months Maurer et al (16) concluded that occlusion rate for segments which were stenosed but not totally occluded at the first examination was 8% for ungrafted arteries (mean interval 11 7 months) The total progression rate (the sum of segments progressing to total occlusion or showing a significant increase in percentage stenosis) was somewhat higher (23%) Glassman et al (9) reported that progression or new lesions were found in 11% of unoperated arteries with an average interval between the two arteriograms of 9.3 months Ben Zvi et al (3) found progression of disease in non-grafted coronary arteries in 6% of surgically treated patients after an average observation period of 10 1 months

Follow up studies of patients with coronary artery disease with a significantly longer interval between arteriograms than in our study give a far higher rate of progression than found by us Bruschke et al (5) reported that 8 of 20 patients with persistent symptoms showed progression or development of narrowings when reexamined 10–88 months after the first examination Ben Zvi et al (3) found that 9 (56%) of 16 medically treated patients with coronary artery disease were found to have progression of lesions in repeat studies carried out after a mean interval of 27 months Their patients had persistent or progressive symptomatology Henderson and Rowe (11) found clear progression in ungrafted arteries in 19 (33%) of 57 patients restudied 2 years after bypass surgery

The interval between arteriograms was significantly longer and the serum cholesterol significantly higher in patients with progression In patients without bypass graft surgery Gensini et al (7) found that 45% of the arteries showed progression when the patients were followed for an average of 26 months Mark et al (15) found progression in 14 of 25 patients with arteriosclerotic coronary artery disease reexamined 5 weeks–72 months after the first coronary angiography They were unable to demonstrate any correlation between progression of angina pectoris or serum lipid abnormalities and the progression of coronary lesions Bemis et al (2) demonstrated significant progression of coronary artery disease in 52% of 73 patients who had two coronary angiograms at intervals of 2–75 months Plasma lipid abnormalities were significantly associated with subsequent arteriographic progression Interval myocardial infarction was associated with evidence of progression but not when the patient had worsening angina without proved myocardial infarction

It is logical that atherosclerotic obstructive coronary artery disease should progress with time and support for this is provided by the significantly longer interval between arteriograms in patient groups with fairly definite arteriographic progression than in those with little or no progression But it also seems clear that the rate of progression differs randomly between patients as emphasized by Bemis et al (2) Lipid abnormalities and interval myocardial infarction both appear to be associated with a high rate of progression while continued but unchanged angina pectoris without myocardial infarction is less predictive of progression

It is known that progression of occlusive coronary artery disease is associated with a deterioration of cardiac function (3) while absence of progression is associated with stable left ventricular function In the present study therefore the stable cardiac function found when the results from the first and second catheterizations were compared could be anticipated as most patients showed no progression of their obstructive coronary artery disease

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Long-term Treatment with Methenamine Hippurate in Recurrent Urinary Tract Infection

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ABSTRACT Twenty four patients with a history of recurrent urinary tract infection and in whom residual urine was considered to be a factor of importance for chronicity, have been treated for an average of 16 months with 1 g methenamine hippurate morning and evening No patient had urinary calculus at the commencement of treatment and neither did any patient have an indwelling catheter In patients without urinary tract infection or in whom abacteriuria was achieved with methenamine hippurate, the number of reinfections was reduced by approximately two thirds compared to periods prior to treatment No patient was completely free from infection throughout the whole treatment period However, in no case did bacteria with extensive resistance appear When urinary tract infection was treated with methenamine hippurate, abacteriuria was achieved in only 6 of 14 patients It would therefore seem that this agent is only of limited value for treatment of established infection In the event of manifest infection it would appear appropriate to treat the infection primarily with antibiotics and to use methenamine hippurate for prophylaxis when abacteriuria has been achieved No patient developed urinary calculus during treatment with methenamine hippurate and no deterioration of renal function or haematological change was observed

The value of long term treatment with antibiotics or chemotherapeutic agents in chronic pyelonephritis and urinary tract infections in patients with factors giving an increased tendency to recurrent infections has been shown by Orsten (19) Bengtsson et al (5) Freeman et al (8) and others During long term treatment there is a risk of adverse reactions and development of bacterial resistance Patients must therefore be carefully selected for such treatment

Long term treatment for indefinite periods is considered to be indicated in adults with one or more of the following conditions 1) Pyelonephritis with anatomical anomaly or with complications which cannot be corrected by surgical procedures 2) Chronic pyelonephritis with bacteriuria and reduced renal function 3) Neurogenic disturbances of bladder function with complicating urinary tract infection

The indications for long term treatment of urinary tract infection in children have been discussed by Agnell et al (1) who recommend long term treatment even in uncomplicated situations in patients with an established tendency to frequent recurrences Sulphonamides nitrofurantoin and ampicillin are mainly used for long term therapy at present All of these drugs can cause side-effects as well as allergic reactions and development of bacterial resistance A long established principle for treatment of urinary tract infections is acidification of the urine This principle has attracted renewed interest as a result of reports that the hippuric acid salt of methenamine (hexamethylenetetramine hippurate) has given good results in both acute and chronic urinary tract infections (3 4 17) and in prophylaxis in connection with instrumental investigations (2)

The aim of the present investigation was to study the treatment and long term prophylaxis of urinary tract infections with methenamine hippurate (Hiprex® Minnesota 3 M Laboratories Ltd Loughborough Leicestersh England)

PHARMACOLOGY

Methenamine hippurate is the salt of methenamine and hippuric acid It is absorbed as such in the gastrointestinal tract circulates unchanged in all body fluids and is rapidly excreted in the urine The substance dissociates in the urine to form hippuric acid and methenamine (18)

Table I *Clinical diagnosis and residual urine volume in 24 patients treated with methenamine hippurate*

Diagnosis	N	Residual urine volume (ml)		
		20–50	51–100	>100
Neurogenic bladder	12	2	5	5
Bladder neck sclerosis with/without prostatic adenoma	3	1	2	
Bladder neck sclerosis and Chronic pyelonephritis	4	4		
Vesico ureteral reflux and Chronic pyelonephritis	5	4	1	

Both parts of the molecule contribute to its antibacterial action Hippuric acid reduces the pH of the urine and thus exerts a bacteriostatic effect If the urine is acidic formaldehyde is slowly released (14) The concentration of formaldehyde is probably only bacteriostatic rather than bacteriocidal (11) The optimum antibacterial effect is obtained at pH values below 5 5 After oral administration of 1 g methenamine hippurate (Hiprex®) the metabolites of the substance can be demonstrated in the urine ithin 30 min and more than 90% of methenamine is ex 1 within 24 hours in subjects with normal renal i (16)

MATERIAL AND METHODS

Twenty four patients (18 men and 6 women) with a mean age of 51 years (range 26–67) and a documented history of recurrent urinary tract infections (clinical symptoms and significant bacteriuria 2–9 times per year during the 2–3 years prior to commencement of the study) were selected for the investigation All patients were known to have a residual urine volume of varying extent (Table I) This was considered to be a significant factor of chronicity Renal function was assessed from serum creatinine (<2 5 mg/100 ml in all patients) and creatinine clearance (>40 ml/min in all patients) I v pyelography micturition urethrocystography and cystourethroscopy were performed in all patients The clinical diagnoses are shown in Table I No patient had an indwelling catheter vesical ureteral or renal calculi at the beginning of the study

Culture of urinary bacteria was performed prior to the study during the study in the event of positive nitrite test and after completion of the investigation The lower limit for significant bacteriuria was placed at 100000 bacteria/ml (13) Every 14 days the morning urine was examined for nitrite glucose and protein and its pH value was determined (BM test 4 Imeco)

The patients received 1 g mehtenamine hippurate (Hiprex®) morning and evening throughout the period of the trial The mean duration of the trial was 16 months (range 12–19) and the mean duration of treatment did not vary between the groups investigated

When significant bacteriuria was found sensitivity tests were performed and Hiprex® replaced by antibiotics according to the results of the sensitivity tests Antibiotic treatment was continued for 3 weeks or until bacteriuria was no longer present whereupon treatment with Hiprex® was resumed

GOT GPT Hb WBC and thrombocyte count were determined prior to the trial and again after 6 months and after completion of the trial

RESULTS

In 10 patients (5 neurogenic bladder disturbances 2 sclerosis of the bladder neck 3 chronic pyelonephritis and sclerosis of the bladder neck) there was no significant bacteriuria prior to the trial In all cases bacteriuria occurred on one occasion during the observation period and in 4 on two occasions Upon culture the urine was found on all occasions to contain coliform bacteria the same organism still being present upon renewed culture one week later during continued treatment with Hiprex® During episodes of bacteriuria sulphonamides alone or combined with ampicillin were given for 3 weeks or until bacteriuria was no longer present whereafter treatment with Hiprex® was resumed

The total number of months of observation for this group was 166 Antibiotic therapy was given for a total of 7 months The same patients had been observed prior to commencement of the study for a total period of 144 months during which treatment for bacteriuria had been given on 40 occasions of about 1 month s duration The ratio for the number of months of infection/number of infection free months during the period of treatment with Hiprex® is thus 0 04 compared to 0 39 for the period prior to treatment with Hiprex®

Fourteen patients had significant bacteriuria at the commencement of the study (13 with coliform bacteria and one with Proteus) In 6 patients with coliform bacteria (5 with vesico-ureteral reflux and chronic pyelonephritis and one with a neurogenic bladder disturbance) abacteriuria developed during treatment with Hiprex® but during the study 2 of them developed significant bacteriuria on one occasion and 2 on two occasions These 6 patients were treated with Hiprex® for a total period of 93 months The ratio for the number of months with

/without bacteriuria was 0 13 for the period of treatment with Hiprex® and 0 3 for the period prior to treatment with Hiprex® In all periods of bacteriuria coliform bacteria were present and treatment was given as described above

Five of the patients with primary coli infection became free from significant bacteriuria after the initial antibiotic therapy Subsequently they developed bacteriuria due to Klebsiella during treatment with Hiprex® Three of them became free from significant bacteriuria after prolonged antibiotic treatment and did not suffer recurrence of infection during the subsequent period of treatment with Hiprex® Two of them were free from bacteria for brief periods only in association with antibiotic treatment Klebsiella infection being present between these periods All these 5 patients with Klebsiella infection also had neurogenic bladder disturbances with residual urine of about 100 ml on repeated determinations

In 2 patients with primary coli infection growth of Klebsiella followed by Proteus developed during the period of observation One of these patients had a neurogenic bladder disturbance and the other sclerosis of the bladder neck

The patient who had a Proteus infection became free from bacteria for a brief period following antibiotic therapy but the same organism was found repeatedly after discontinuation of the treatment Throughout the period of observation however the patient remained free from acute exacerbation of his chronic pyelonephritis The 3 patients who had Proteus infections at the final examination all had multiple vesical diverticula but no demonstrable vesico ureteral reflux

Bacterial typing in connection with bacteriuria was not performed and it was therefore not possible to determine whether recurrence or reinfection had occurred During treatment with Hiprex® the pH of the urine was 5 0–5 6 as long as abacteriuria persisted during bacteriuria it rose to 5 8–7 2 In no patient did calculus develop in the urinary tract during the observation period No signs of alteration of liver or renal function were found during the period of observation nor were haematological changes observed

Two patients with a previous history of peptic ulcer complained of transient epigastralgia when taking Hiprex® on an empty stomach The symptoms disappeared when the product was taken with meals

DISCUSSION

In some patients there is a pronounced tendency to recurrence of urinary tract infection These recurrences are sometimes due to reinfection with another organism but sometimes especially in the presence of chronicity factors such as calculus or residual urine infection recurs with the same organism Antibiotics such as ampicillin and tetracycline also influence the bacterial strains of the bowel with development of resistant strains in the gut flora and increased risk of urinary reinfections

The value of long term treatment in urinary tract infections has been demonstrated in large studies Örsten et al (20) presented the results of long term treatment of 306 patients with chronic pyelonephritis and urinary tract infection They found that the risk of recurrence was reduced during long term treatment from 60% to 20% For several reasons long term therapy should only be given upon strict indications Previously cyclic long term treatment with several drugs or intermittent treatment was given but since these modes of therapy did not give as good results as continuous treatment with the same drug in a constant dosage these forms of treatment are nowadays little used For long term therapy a drug with low toxicity is required which gives constant and adequate antibacterial levels and little risk of the development of resistant strains not controlled with other chemotherapeutic agents

The bacteriostatic agents suitable for long term treatment are sulphonamides nitrofurantoin and erythromycin Ampicillin and oxacillin are bacteriocidal agents suitable for long term use All of these drugs may cause hypersensitivity reactions and development of resistant bacterial strains

Healthy individuals have a not considerable self defence against urinary tract infections as their urine has antibacterial activity (12) which is correlated to osmolality and urea and ammonium concentration At pH values between 6 5 and 5 0 the antibacterial activity of the urine increases with decreasing pH A precondition for the achievement of a low pH is that the acidifying capacity of the kidneys is retained which is not always the case in chronic pyelonephritis

The established principle of acidifying the urine has attracted new interest with the introduction of methenamine hippurate This product has given good results in the prophylaxis of infection in connection with instrumental investigations of the

urethra and bladder (2) and has also been investigated in long term therapy. Bengtsson and Orsten (6) obtained freedom from bacteria in 59% of 58 patients with various conditions by treatment for 3 weeks – 7 months. The poorest results were obtained in chronic pyelonephritis with complicating factors such as papillitis necroticans or diabetes mellitus. In geriatric patients long term treatment with methenamine hippurate gave freedom from bacteria in 83% (3). In the presence of chronicity factors such as calculus and pyelonephritis freedom from bacteria was achieved in 40–60% of the patients reported by Pearl and Burch (15), Gerstein et al. (9) and Gibson (10). Reinfection or recurrence of infection generally occurred in these patients shortly after discontinuation of the antibacterial treatment.

In the present study treatment with methenamine hippurate was evaluated in a number of patients with a documented history of pronounced tendency to recurrence of urinary tract infections. In all patients there was an association between tendency to recurrence of infection and residual urine. Upon reduction of urinary pH to values below 5.6 the number of recurrences was reduced in [illegible] patients compared to periods without treatment. [illegible] no patient was free from significant bac[illegible]ria throughout the whole period of treatment. [illegible]he number of periods of infection was reduced during treatment with Hiprex® for patients without bacteriuria from 40 periods during 144 months to 14 periods during 166 months, i.e. an approximate reduction of the number of infection periods to one fourth.

The possibilities of treating a manifest infection in patients with residual urine by means of methenamine hippurate appear to be limited since abacteriuria was achieved in only 6 of 14 patients. One of these patients had a Proteus infection; however, it could hardly be expected that methenamine hippurate would completely eliminate a urease producing organism. Casselman (7) and Gerstein et al. (9) have previously reported that methenamine hippurate is not able to maintain sterile urine in patients with indwelling catheters.

The 6 patients who became free from significant bacteriuria with Hiprex® suffered a total of 8 recurrences during continued treatment with Hiprex®, which is in agreement with the incidence of recurrence in patients who were primarily free from bacteria. The number of recurrences in these 6 patients was reduced by about two thirds during treatment with methenamine hippurate.

In no patient with primary abacteriuria or in whom manifest infection disappeared did bacterial strains resistant to sulphonamides or ampicillin develop during treatment with Hiprex®.

Antibiotics are often given even in the absence of clinical signs of infection to patients with a confirmed tendency to recurrence of urinary tract infection and with predisposing factors such as chronic pyelonephritis with calculus, vesical diverticula or indwelling catheters. As a result of this practice resistant Proteus and Pseudomonas strains ultimately develop in a not inconsiderable proportion of these patients. It is desirable to reduce the use of antibiotics in this group of patients without increasing the risk of complications and further impairment of renal function. Methenamine hippurate would seem to offer an alternative to antibiotics. This substance does not eliminate the risk of bacteriuria but reduces the number of recurrences. The infections which developed during treatment with methenamine hippurate in the present study were readily dealt with by conventional treatment in most cases.

Methenamine hippurate is of limited value for the treatment of established urinary tract infections. It would seem appropriate to initiate treatment with antibiotics and only when the bacteriuria has disappeared to give methenamine hippurate in an attempt to reduce the risk of recurrence.

When urine is infected with Proteus and some strains of Pseudomonas, Escherichia coli and Klebsiella, hyperammoniuria and alkalinity develop. Methenamine hippurate cannot be used to eliminate urease producing bacteria but the precipitation of magnesium ammonium phosphate (struvit) and calcium phosphate favoured by these bacteria may be reduced, as in no patient in this study did urinary calculus develop. The addition of methenamine hippurate may retard the progressive alkalinization of urine due to growth of ureaseproducing Proteus. This effect is probably doserelated and also related to the size of the bacterial inoculum. Since methenamine hippurate prevents a rise in urinary pH, either by exerting a bacteriostatic action or by blocking bacterial urease, precipitation of salts may likewise be prevented. These properties of methenamine hippurate may be of potential value in the treatment of urinary tract infection accompanied by stone formation.

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Myxoedema Coma

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ABSTRACT Myxoedema coma is a medical emergency which must be treated immediately Otherwise the mortality is high It is important to administer L triiodothyronine and corticosteroids early If hypoventilation occurs, artificial respiration may be necessary In the two cases described here the outcome was successful probably owing to quick substitution The causes of myxoedema and the symptomatology are discussed

Myxoedema coma is a rare complication of myxoedema It is a medical emergency which must be treated in an intensive care unit with active substitution The first case was reported as long ago as 1879 from St Thomas Hospital London but it was not until 1953 that the next one was presented by Summers and le Marquand Hemsted Hausmann By 1964 Leon Sotomayor (6) had collected 131 cases 4 of them his own Since then several other cases have been reported some of them from Scandinavia (5 8) Myxoedema is four times more common in females than in males and the average age of patients is between 65 and 75

CASE REPORTS

Case 1

A 57 year-old female In the summer of 1973 she had had thyreotoxicosis and had been treated in Oct 1973 with a single dose of 6 mCi radioactive iodine (^{131}I) Early in 1974 she had felt still colder was putting on weight and had also been confused with periods of lethargy At a revisit to the Clinic of Radiotherapy in Feb 1974 she had a PBI of 0 8 μg/100 ml no ^{131}I uptake by the thyroid could be registered Finally she was not able to get out of bed

She was admitted acutely to the Medical Department on March 20th and treatment with L thyroxine 0 05 mg×1 was started BP 140/80 mmHg pulse rate 80/min On March 22nd she had short periods of unconsciousness

On March 23rd she had a period of pulselessness and no measurable BP Body temperature 35 9°C pulse rate 48 systolic BP 60 ECG bigeminy β methasone 4 mg×2 and L thyroxine 0 05 mg×1 were given On March 24th the body temperature was 36 5°C BP 160/95 and pulse rate 80 Medication as the day before The patient was confused aggressive and had a hyperactive behaviour On March 25th the body temperature was 37 2°C BP 145/80 and pulse rate 55 The same doses of β-methasone and L thyroxine were given again At intervals the patient became mentally more lucid but was agitated On March 29th she became paranoic Perphenazine treatment was initiated and the L thyroxine dose was increased to 0 1 mg×1

On April 14th the patient was completely lucid calm and balanced The puffiness of face and hands was reduced as was the weight

Laboratory data Hb ESR and WBC normal Serum electrolytes potassium 3 6–4 2 sodium 138 chloride 104–99 mEq/l Cholesterol 378–216 mg/100 ml PBI 0 5 μg/100 ml T_3 15% 4 weeks later PBI 8 0 μg/100 ml Serum creatinine 1 3–1 0 mg/100 ml Urine contained initially 8–10 erythrocytes/field later normalized ANF negative ECG sinus bradycardia. At later visits the patient has been euthyroid

Case 2

A 30-year-old female treated by a gynaecologist for 3 years because of amenorrhoea No hormone investigations were made In recent months she had become increasingly tired and pale and had felt cold For the last few days before admission she had been confined to bed

She was admitted on April 4th at 7 30 a m with a grand mal seizure She was unconscious had a body temperature of 33°C and hypoglycaemia—14 mg/100 ml Pulse rate 48/min BP 80/70 mmHg The heart sounds were faint ECG bradycardia T wave inversion low voltage Blood gas analysis showed increased pCO_2 Electrolytes Na 120 Cl 95 HCO_3 19.5 At 10 a m L triiodothyronine 100 μg i v and β methasone 4 mg i v were given together with peroral L thyroxine 0 05 mg×1 and nasal oxygen At 1 p m BP was 100 body temperature 35 6°C pulse rate 62 Conscious At 10 p m L triiodothyronine 50 μg and β-methasone 4 mg were given The blood gases had normalized

On April 26th the body temperature was 36 8°C pulse rate 96 BP 105/80 and the patient was completely lucid and awake Treatment was changed to L-triiodothyronine 20 μg×3 L thyroxine 0 05 mg×1 and β-methasone 4 mg×2 orally Electrolytes had normalized On

April 28th L-triiodothyronine was decreased to 20 μg×2 and β-methasone administration was discontinued After another 2 days the L-triiodothyronine dose was decreased to 20 μg ½×1 and discontinued after a further 2 days At the same time the L-thyroxine dose was increased to 0 1 mg Chest X-ray heart volume 290 cm³/m²

Laboratory data Hb 8 4–10 5–9 g/100 ml WBC 3 300–8 200/mm³ platelets 130 000/mm³ ESR 19 mm/h Serum creatinine 1 7–1 5–1 3 mg/100 ml Good urine output Thymol 0 3–1 7 U GPT 90–118–94–41 IU Serum bilirubin normal Serum alkaline phosphatase 4 6–6 0–4 7 IU GOT 265–370–140–44 IU Sodium 120–142 potassium 3.3–4.5 standard bicarbonate 19 5 and chloride 95–110 mEq/l Serum iron 38 μg/100 ml TIBC 226 μg/100 ml Serum folic acid 3 4 Cholesterol 242 mg/100 ml Urine deposit normal uric acid 4 6 mg/100 ml PBI 0 5–10 2 μg/100 ml T_3 26 7–21 0%

COMMENTS

Myxoedema is sometimes seen after thyroidectomy or after treatment with ^{131}I It can occur in patients with subclinical autoimmune thyroiditis and a high ANF titer Myxoedema may be due to a primary disorder of the thyroid gland or it may be secondary to pituitary failure Tertiary hypothyroidism is conditioned by a hypothalamus disorder Coma is the terminal stage of myxoedema Primary features unconsciousness and hypothermia Other symp- are hypoventilation hypotension fluid and electrolyte imbalance and peripheral vascular collapse Decreased alveolar ventilation gives CO_2 retention which induces CO_2 narcosis and respiratory acidosis This is caused by depression of the respiratory center interference with the nervous transmission of respiratory musculature reduced contractility in respiratory musculature and alveolar capillary block (1 3 4 9)

Coma appearing in a formerly hypothyroid patient induced by sedatives or narcosis needs assisted respiration until the effects of the drugs or the anaesthesia have disappeared The changes of the upper respiratory tract can be seen as an extreme swelling e g mucopolysaccharide deposition of the tongue During coma the tongue may fall back and obstruct upper airways In addition to myxoedematous infiltrations in the respiratory musculature obesity emphysema pulmonary congestion and upper respiratory tract infection can diminish the ventilatory capacity (3) Sedatives narcosis and minor tranquilizers also decrease the sensibility of the respiratory center

The total body store of sodium is high in hypothyroid patients but patients with myxoedema coma may present hyponatraemia Total body water and urine sodium are increased but the glomerular filtration rate and renal plasma flow are reduced The cause of the diminished diuresis may be renal mucopolysaccharide deposition Urine osmolarity is increased and plasma osmolarity reduced The balance between the intra and extracellular space is disturbed The exact mechanism is not clear Lack of thyroid hormone reduced cardiac output and in some cases a low utilization of antidiuretic hormone have been discussed (10)

Plasma volume is reduced but the extracellular volume is increased The oedema is not pitting and serous exudate may occur After thyroid hormone treatment total water is reduced and serum sodium increases Fluid restrictions are obligatory but hypertonic infusions are not usually required (2 4 5 7 9) Serum potassium may be high or low The combination of low sodium and high potassium suggests adrenal insufficiency but most patients with myxoedema coma have normal 17 OH steroids ACTH stimulation test is normal There may even be hypocalcaemia especially in thyroidectomized patients in whom the parathyroid glands have been removed as well High pCO_2 results in low plasma Ca The increased capillary permeability implies that the exudate has a higher protein content than that of patients with heart failure (10) Enlargement of the cardiac silhouette can be seen on the chest X-ray and this has been attributed to increased fluids in the pericardium which is also mucopolysaccharidic Fluids in the pleural space are also common

The cardiac output and stroke volume are reduced as is the circulatory time There is a high risk of coronary arteriosclerosis in hypothyroid patients especially in those with myxoedema coma due to mycocardial deposition of mycopolysaccharides and hypercholesterolaemia. Extreme bradycardia, different types of heart block low voltage prolongation of the QT segment and T wave inversion may occur in the ECG Hypotension is also common and the low BP will not respond to vasopressor agents unless thyroid hormone and steroids are added (1 2)

In myxoedema coma there may be adrenal hypofunction but plasma cortisol is normal The cause is decreased ACTH outflow due to the elevated TSH secretion (4 10) In primary myxoedema the most common type TSH is increased In

secondary myxoedema TSH is unchanged due to pituitary insufficiency Thyrotropin releasing hormone (TRH) may be suitable for clinical use Administration of TRH to patients with primary myxoedema results in a greater rise of TSH than in those with pituitary deficiency The role of aldosterone is unknown Hypothermia is alarming and suggests a serious metabolic state It disappears after substitution External warming may result in a peripheral vascular collapse and increased O_2 consumption and vascular collapse (1 10) Hypoglycaemia may occur in myxoedema coma with a prolonged hypothermia or with concomitant adrenal insufficiency

The intestinal glucose absorption is decreased and the mobilization of liver glucogen is insufficient (1 4 5 10) The external nervous system shows various complications The intelligence is often lowered with cretinism Amnesia emotional changes somnolence depressions and paranoic compulsions may occur The dysfunction may be caused by decreased respiration and CO_2 narcosis *hyponatraemia and cerebral oedema* lack of thyroid hormone or hypoglycaemia (1) One patient in four has grand mal seizures

The contraction and relaxation of muscles are slow The muscle volume is increased This results in slowed responses in the reflex arch and increases of CPK GOT and LDH (1) This condition normalizes when the hormone balance is restored but the cause is unknown Obstipation and gas in the intestine are often symptoms of hypothyroidism In myxoedema coma paralytic ileus may occur (1) This state is caused by lost elasticity and deficient peristalsis The state is improved after *substitution There may even be urinary retention* Cachexia occurs often when the patient is seriously ill and the food intake is much reduced Increased capillary fragility is common and may result in haemorrhage in the gastrointestinal tract and the skin Anoxia may have the same consequences *Bone marrow depressions and low platelet count* may be seen The prognosis is poor Earlier the mortality was 80% but has now decreased to 50%

Provoking factors

Exposure to cold may result in coma Most of the patients fall ill during the cold time of the year (4) Phenothiazines and imipramine may result in coma as may narcosis and barbiturates Infections traumas and operations are stressful conditions which may precipitate the development of coma

Treatment

Substitution therapy with thyroid preparations in myxoedema coma has been given in various ways (4 5) L thyroxine starts to take effect after 3 or 4 days and has its maximal effect within 15 days *L triiodothyronine takes effect within 5 hours and* has its maximal effect after 24–36 hours L triiodothyronine for parenteral administration is now available (Erco) It can even be prepared by dissolving L triiodothyronine in 0 1% natrium hydroxide whereupon distilled water and 10% NaCl are added so that the concentration is 0 85% NaCl 0 05% NaOH and 30 µg L-triiodothyronine/ml (5) A suitable dose is considered to be 100 µg i v or by gastric tube every 12 hours or 50 µg every 6 hours until body temperature rises and consciousness is regained (4) Another suggestion is 0 10 mg L triiodothyronine in 1 000 ml 5% glucose i v The subsequent change to more long acting *L thyroxine must be gradual In coronary sclerosis* and angina pectoris it is necessary to be careful with initial substitution

During thyroid hormone substitution the pituitary adrenal axis does not return to normal function fast enough to meet the increased cortisol demand (4 5 10) Therefore hydrocortisone in doses of 50–100 mg or 25 mg prednisolone is given every 6 hours for 3–5 days Artificial respiration may be necessary because of CO_2 retention Oxygen gas is often required and sometimes tracheostomy (8) Too quick warming must be avoided owing to the risk of vasodilation and peripheral vascular collapse The i v fluid supply must be restricted on *account of the risk of overloading Glucose* perhaps with NaCl added can be given to correct possible hypoglycaemia Prophylactic treatment with antibiotics is recommended as there is a high frequency of silent infections Cardiac arrhythmias and heart failure are treated as usual

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The Degree of Bone Mineralization in Chronic Renal Failure Estimated from Phosphorus/Hydroxyproline Ratio in Bone Biopsies

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ABSTRACT *The degree of bone mineralization is* the proportion between the contents of mineral and collagen in bone As phosphorus (P) and hydroxy proline (Hypro) constitute a constant fraction of bone mineral and collagen, respectively, the P/Hypro ratio in bone tissue provides an estimate of the degree of mineralization The P/Hypro ratio has been studied in bone biopsies from 42 patients with severe chronic renal failure A few of them had a subnormal P/Hypro ratio and these patients corresponded to the diagnosis of classical osteomalacia or probably hyperparathyroidism However, the mean bone P/Hypro ratio was significantly higher than normal, indicating a general tendency towards a higher de gree of bone mineralization in patients with chronic renal failure

As phosphorus (P) constitutes an almost constant fraction of the mineral in bone (6 7) the P content of bone reflects the mineral content The amino acid hydroxyproline *(Hypro) constitutes a constant* fraction of collagen and collagen is the only protein that contains Hypro (4) The amount of this amino acid in bone accord ngly reflects the content of col lagen The P/Hypro ratio therefore reflects the min eral/collagen ratio or in other words the degree of mineralization In bone from patients with classical osteomalacia there is a decreased quantity of min eral and a high or normal quantity of collagen (11) consequently a low P/Hypro ratio will be found in these patients The determination of P/Hypro in bone t ssue should therefore yield informat on on the degree of osteomalacia and probably also on the degree of osteosclerosis

From this point of view the bone changes were exam ned in a group of patients with chronic renal failure *Bone disease is a progressive problem for* these patients and the nature of the uremic bone lesion is not yet fully understood (13)

PATIENTS AND METHODS

Forty two patients 30 females and 12 males with chron c renal failure and creatinine clearances of 0-75 ml min (mean 10) were examined Their ages varied from 0 to 74 years (mean 59) Twenty of the patients had chronic pyelonephritis 12 had interst tial nephritis 4 had chron glomerulonephritis and 6 had some other more uncom mon renal disease In most patients the diagnos s was based on renal b opsy The durat on of renal d sease was 1–12 years (mean 9 6)

P/Hypro rat o data from 137 persons without kidney or bone disorder were used as reference values (16) The ages of these subjects were uniformly d stributed from 70 to 89 years (mean 55) *The P/Hypro ratio (mol/mol)* was est mated in bone biopsies obtained from the anterior superior iliac spine in local anaesthesia Th s method has been described earl er (15)

In normal persons without bone disorder the P Hypro ratio in reases sl ghtly w th age (16) We have taken this into account for the pat ents with chronic renal failure by calculat ng the g(P/Hypro) i e the d fference between the pat ent s P/Hypro ratio (x) and the mean for normals of the same age (x_{normal}) d v ded by the standard deviat on for the normal group $S(x_{normal})$ as follows

$$g(P/Hypro) \quad \frac{x \quad x_{norm}}{S(x_{normal})}$$

Th s value expresses how many normal standard devia tions the P/Hypro rat o of a patient with chron c renal failure deviated from the mean value of normal persons of the same age

In add tion we estimated serum alkaline phosphatase (normal≤3 ? Bessey Lowry units (?)) serum phosphorus (normal $x \pm S(x)$=1 70+0 15 mmol/l (9 1?)) and serum calc um (normal $x + S(x)$—4 70+0 40 mEq/l (1))

X ray exam nations of the hands lumbal spine and

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renal failure was greater than normal This may be caused by a greater interindividual variation due to the many different physiological changes in the uremic state but it may also be due to a greater intraindividual variation as histological examinations of the bone of uremic patients often show a mixture of bone changes (9)

A few of the patients had a subnormal P/Hypro ratio but the majority had values higher than normal In this study no attempt was made to divide the patients into groups with different bone diseases However patients with elevated serum alkaline phosphatases had relatively low P/Hypro and patients with subnormal P/Hypro corresponded very well to patients with osteomalacia or possibly hyperparathyroidism

Some of the patients had a very high degree of mineralization Only an insignificant number of these patients had a diagnosis of osteosclerosis at X ray examination However the roentgenological appearance of osteosclerosis depends not only on the degree of mineralization but also on the quantity of bone tissue per volume If the cortex were very thin and the trabeculae very sparse a high degree of mineralization could be found in each trabecula but osteosclerosis would not be expected either at X ray examination or by osteodensitometric measurement

This study did not show an association between the nature of the renal disease or the duration of the uremic state and bone P/Hypro

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Iron Metabolism in Porphyria Cutanea Tarda

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ABSTRACT The iron metabolism has been studied in 11 patients with porphyria cutanea tarda Despite significantly increased amounts of iron in the serum and liver, the porphyria patients absorb not less but significantly more radioiron than normal controls Whereas phlebotomy induced iron deficiency stimulates the absorption in controls, no further stimulation is found in the porphyria patients The plasma iron turnover is high in the porphyria patients and some of this iron is probably deposited in the liver The relationship between the high iron absorption high plasma iron turnover, high liver iron liver damage and uroporphyrin production in the liver is discussed It is suggested that two concurrent genetic defects may cause porphyria cutanea tarda one in iron absorption and one in porphyrin synthesis by damaged liver cells The absorption of inorganic iron was higher than that of hemoglobin iron The possible damage which fortification with inorganic iron could cause to the very small group of porphyria patients is discussed

Iron metabolism in porphyria cutanea tarda has been of clinical interest since the introduction of therapeutic iron depletion by phlebotomy (9 14 21) Such depletion manifestly reduces photosensitivity and uroporphyrin excretion (14) The problem is whether an increase in iron intake increases photosensitivity and uroporphyrin production

An increase in iron fortification of flour is being discussed at present in several countries (3 4 6 10 18 22) The present purpose was therefore to study the intestinal absorption and plasma turnover of different forms of radioiron in porphyria cutanea tarda before and after therapeutic phlebotomy

MATERIAL

Six women and 5 men with porphyria cutanea tarda were examined The unusual predominance of women cannot be explained here The diagnosis was based on the clinical picture porphyrin excretion and the liver function and biopsy The patients are described in Table I Normal materials for the iron ($FeSO_4$) absorption serum iron transferrin and iron plasma clearance have been described (7 8) Comparative values for hemoglobin iron absorption had to be obtained in blood donors since the hemoglobin iron absorption could not be studied until phlebotomy treatment of the porphyria patients had started Blood donors were paired with porphyria patients to be comparable as regards age sex and number of phlebotomies (Table II)

A group of normal persons examined previously for serum iron transferrin and iron absorption was used as a control group in this respect (7)

METHODS

The routine methods and normal values employed in the Central Laboratory of the Karolinska Hospital were used for transaminase hemoglobin hematocrit iron transferrin and porphyrin determinations as well as for bromsulphthalein and galactose load tests and are shown in Table I

Liver biopsy was obtained with a Vim Silverman needle and stained with Prussian blue stain and van Gieson s stain

Four different iron absorption studies were performed 1) The absorption of 0 25 mg $^{59}FeSO_4$ was studied *before* the beginning of phlebotomy treatment in female patients 1 2 and 3 and male patients 7 8 9 and 10 2) The same dose was used to study absorption *after* the beginning of phlebotomy treatment in the same patients 3) The absorption of 3 5 mg iron in the form of ^{59}Fe hemoglobin ($^{59}FeHb$) was studied when patients had been phlebotomized [illegible] times in *female patients* 2 4 5 and 6 and in *male patients* 9 10 and 11 4) Simultaneously in the same patients the absorption of 3 5 mg iron in the form of $^{55}FeSO$ was studied The ^{55}Fe activity per ml red cells was measured and the relative incorporation ($_{ery}{}^{55}FeSO$) calculated on the basis of body weight

Some weeks later a study was performed of the absorption of the same dose of $^{59}FeSO_4$ [illegible] with a whole body counter as described earlier (7 [illegible] statistically significant correlation ($r=0$ 6) was f[illegible]

Table I *Clinical description of the patients*

Pat no	Initial age (y)	Sex	Duration of disease	Alcohol consumption[a]	Liver size[b]	Liver biopsy[c] Fat	Fibrosis	Iron	Transaminases GOT GPT (U)	Bromsulphthalein retention (%)	T ½ of i v galactose load (min)	Urinary uroporphyrins and coproporphyrins[d] (μg/24 h)
Normal values									20 17 11	5	17 2 765	10 100
1	60	♀	1½	++	Normal	++	+	++	17 16	15	n d	614 1 788
2	48	♀	1½	+	Normal	+	–	+	24 37	8	n d	101 4 763
3	62	♀	Appr 5	+++	Normal	–	–	+++	40 23	4 7	n d	384 1 587
4	45	♀	1	++	Enlarged		n d		48 48	n d	n d	537 2 700
5	67	♀	3	+	Normal	+	++	++	74 26	n d	39	390 3 790
6	63	♀	6	+	Normal	++		+	40 27	n d	26	375 3 582
7	66	♂	3	+	Enlarged		n d		37 26	8	n d	175 3 434
8	72	♂	2	+	Enlarged	–	++	–	43 14	n d	15	552 913
9	46	♂	2	++	Normal		n d		28 31	n d	15	84 1 745
10	61	♂	1	+	Normal		n d		49 13	n d	22	159 1 950
11	21	♂	3	+	Normal	–	–	+	18	n d	12	114

[a] – No consumption of alcohol + no abuse ++ previously supervised by temperance board +++ currently supervised emperance board [b] By palpation and X ray [c] +Slight ++ moderate +++ pronounced [d] Most pathologic pair of s quoted n d = not done

measurements of the absorption of 3 5 mg $FeSO_4$ The regression equation

$$_{Abs}{}^{59}FeSO_4 = 0\ 835 \quad _{Ery}{}^{55}FeSO_4 + 7\ 0$$

was used to convert the amount of $^{55}FeSO_4$ incorporated into red cells to a figure for $^{59}FeSO_4$ absorbed In this way figures for the absorption of $^{55}FeSO_4$ and ^{59}Hb on the same day could be obtained

RESULTS

Liver function None of the patients was socially crippled by alcohol but 4/10 had a relatively high consumption Consequently serum transaminases were pathological in 9/11 patients and 6/10 had an increased bromsulphthalein and/or galactose test (Table I)

Porphyrin excretion The local upper normal limit for uroporphyrin excretion is 10 μg/24 h All patients had excretions which were increased 90–475 times Coproporphyrin excretion was normal in 2/11 patients and increased up to 7 times in 9/11

Iron metabolism before phlebotomy Hemoglobin and hematocrit values were normal The mean serum iron was significantly higher (0 001>p) than in controls (7) Since the total iron binding capacity did not differ significantly from control values the transferrin saturation with iron was also pathologically high (Table III)

The mean plasma iron clearance rate was within normal limits This implies together with the high serum iron concentration that the mean plasma iron turnover (mg iron/min) is higher than the upper normal level (Table III)

The mean absorption of ferrous iron was significantly higher (0 5>p>0 01) in the male patients than in the male controls (Table IV) No significance was found regarding the three female patients but they too had numerically quite high absorption values

Iron metabolism after phlebotomy Hemoglobin values decreased after phlebotomy in each of the 6 patients studied as did the serum iron and the

Table II *Hemoglobin iron absorption in phlebotomi ed patients with porphyria cutanea tarda compared to phlebotomi ed healthy blood donors*

	Pats	Blood donors
No of subj	7	7
Blood donations in 3 y prior to study (mean and range)	15 (3–22)	
Age (y)	21–67	21–63
Sex	4♀ 3♂	3♀ 4♂
Absorp on (% of 3 45 mg iron dose means + S E M)		
$^{59}FeSO_4$	29 4+10 7	30 3+1? 7
$^{55}FeSO_4$	31 7+6 5	27 7+7 4
^{59}FeHb	16 8+2 8	17 1+4 ?
Mean of ind vidual differences between $FeSO_4$ and Hb iron absorpt on	14 9+5 05	10 6+6 45
Statistical s gnif cance of difference	0 05<p<0 01	Not significant

Table III *Effect of phlebotomy treatment on blood values and iron metabolism in porphyria patients (mean±S E M)*

	Before phlebotomies	After phlebotomies
Hb (g/100 ml)	15 3+0 5	13 0+1 0
Serum iron (mg/100 ml)[a]	0 ?10+0 023	0 123+0 018
Total iron b nd ng capacity (mg Fe/100 ml)[a]	0 332+0 016	0 384+0 038
Transferrin saturation (%)	64+7	31+8
$^{59}Fe^+$ plasma clearance *rate(T 1/? m n)*[b]	*119+10*	91+17
Plasma iron turnover rate (mg/m n)	0 0?4+0 001	0 0?3+0 008
Iron absorption (% of 0 25 mg dose)	44 8+9 7	53 2+10 ?

Previously publ shed control values (7)

[b] Previously publ shed control values (8)

[c] Calculated as (serum iron) (assumed ? 75 l plasma volume) (% ^{59}Fe cleared/m n) 0 0?4 mg/min correspond to 47 5 mg/24 h Normal values ?0–42 mg/?4 h

transferrin saturation with iron (4 patients) The total iron binding capacity increased in all 4 patients studied Statistically the decrease in mean serum iron was signif cant (0 0?>p>0 01) (Table III)

The plasma iron clearance rate increased after phlebotomy in all patients but two Since a moderate but statistically insignificant acceleration of the plasma iron clearance rate was accompanied by a sign ficant decrease in the serum iron concentration no significant change was found in the average plasma iron turnover (Table III)

The mean iron absorpt on d d not change s g n f cantly The d fference between the mean absorpt on before and after phlebotomy was not statistically significant neither d d the mean of the individual differences between absorptions after and before phlebotomy differ s gnificantly from zero

Absorption of inorganic iron ($FeSO_4$) as con pared to an organic iron complex (hemoglobin) Unfortunately no values are available prior to the start of phlebotomy treatment After starting the treatment the mean absorpt on of hemoglob n iron in the porphyria patients was almost *identical to* that in the matched blood donors who had given comparable amounts of blood (Table II) However the mean of the ind vidual differences between hemoglob n iron and $FeSO_4$ absorption differs almost significantly (0 05>p>0 01) from zero for the porphyria pat ents but not for the controls (Table II)

DISCUSSION

If in porphyria cutanea tarda iron absorption regulation functioned normally the pat ents would with their massive amounts of iron in the bone marrow liver and serum absorb less than normal controls and their absorption would increase after phlebotomy induced iron deficiency (8) Neither is true Instead the male porphyria patients desp te their large iron stores absorb more than controls and their absorption in contrast to that of controls is not further st mulated by phlebotomy It is suggested therefore that the iron absorpt on regulat on is defective in porphyria cutanea tarda which explains the high serum iron and transferrin saturat on

There are obviously at least two mechanisms wh ch are clearly pathological in porphyria cutanea

Table IV *Iron absorption (% of 0.25 mg Fe dose) in non pl lebotor i ed porphyr a cutanea tarda patients (means±S E M)*

	$FeSO_4$ (0 25 mg Fe)	
	Men	Women
Porphyria patients	28 7+? 4	66 ?+?1 ?
N	4	3
Prev ously publ shed controls (7)	19 0+? 3	39 7+4 5
N	?4	?7

A a

tarda Firstly practically all patients had liver disease Secondly the regulation of iron absorption seems to be defective

It is difficult to establish the cause and the course of clinical events in porphyria cutanea tarda It has been suggested that large amounts of parenchymal iron may contribute to the production of liver damage (2 5) and an increased intestinal iron absorption could of course lead to large amounts of iron in the liver The finding of an increased plasma iron turnover in porphyria cutanea tarda also suggests that extra iron is deposited in the body probably mainly in the liver Thus increased deposition of *iron in the liver could be suspected of being a con*tributory cause of porphyria cutanea tarda

However many patients without the disposition for porphyria cutanea tarda can develop comparable degrees of liver damage without developing porphyria (11 12 16 17 19 20) A few porphyria patients have normal iron stores (14) Even in descriptions of primary idiopathic hemochromatosis a genetic defect in iron absorption regulation porphyria is usually not mentioned (1) The possibility must therefore be considered that this form of porphyria could be the result of two combined genetic defects one in iron absorption regulation ding to iron accumulation and liver damage and other in porphyrin formation This hypothesis is tradicted neither by the similarity in age at which porphyria and hemochromatosis patients present symptoms when enough iron has been accumulated to cause liver damage nor by the pre*dominance of men in both disease groups It is also* supported by the clinical effect of phlebotomy treatment as reviewed by Lundvall et al (14) and by *the clinical relapses after iron therapy* (13)

On the other hand it is also conceivable that the pathogenetic chain of events starts in the liver and pancreas rather than in the intestine and that the high iron absorption rather than causing liver damage may be a result of hepatopancreatic insufficiency An increased iron absorption in pancreatogenous steatorrhea is sometimes seen However since steatorrhea or pancreatic disease are rare in patients with porphyria cutanea tarda or idiopathic hemochromatosis and since the liver insufficiency was only moderate in the present patients the first hypothesis is believed to be more plausible

The absorption of hemoglobin iron was shown to be lower than that of inorganic iron in the present porphyria patients and it is improbable that the increased iron stores are derived from heme iron in the diet

Fairly large and increasing amounts of inorganic iron are added to food for fortification purposes at the present time Assuming a daily intake of 10 mg fortification iron per day (15) and assuming that in agreement with the present figures 2 mg of this are absorbed 7 3 g of iron could be deposited probably largely in the liver during a 10-year period As previously pointed out by Crosby (3) this is probably an amount sufficient to be toxic for the predisposed liver

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On Fibrinolysis in Diabetes Mellitus

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Abstract In diabetics irrespective of age at onset and standing of the disease the fibrinolytic activity in the blood is decreased most markedly in obese patients and in those with angiopathy In this investigation we found —significantly more often than in controls—a low release of the plasminogen activator to the blood from the endothelial cells and also in one fourth of the diabetics an abnormally low activator activity of the vessel walls These changes as well as the significantly elevated levels of inhibitors of the fibrinolytic activity will impair removal of fibrin deposits and possibly contribute to the development of vascular lesions

Lesions of small and large vessels are the most dreaded complications in diabetes of long standing Ditzel (11) reported that death from myocardial infarction was 2–3 times more common and death from nephropathy 17 times more common among diabetics than in a comparable non diabetic group Since such changes are vascular it was thought worthwhile to study the coagulative and fibrinolytic systems in diabetes Most investigators have found coagulation factors to be increased but there is no such agreement about the fibrinolytic system some workers having found an abnormally low (6 12 13) and others an abnormally high (7 10) fibrinolytic activity Almost all available reports concern only the spontaneous fibrinolytic activity More elaborate methods have now made it possible to measure the plasminogen activator activity of the vessel walls (23) and thereby assess the endothelial activator content The biopsy specimens used in such methods are taken from superficial veins in the dorsum of the hand The activator activity of these veins varies closely with that of the temporal arteries (5)

Moreover methods are now available for estimation of the fibrinolytic capacity i e the maximal release of activator from the endothelium to the blood stream in response to an exogenous stimulus Various kinds of stimulation have been used such as exercise adrenalin i v and venous occlusion Venous occlusion for 20 min at a pressure half way between the diastolic and systolic BP has proved most useful (25)

We have found (4) the fibrinolytic capacity in long standing diabetes (more than 10 years) to be significantly lower in the presence than in the absence of microangiopathy (retinopathy)

We have also found (1) a negative correlation between the degree of overweight on the one hand and the spontaneous fibrinolytic activity as well as the fibrinolytic capacity on the other A significant correlation has also been demonstrated between the degree of overweight and the plasminogen activator activity of the vessel walls in diabetics (1) and in non-diabetics (2)

This paper reports an investigation of the fibrinolytic system in diabetics for any variation with the duration of the disease with age at onset of diabetes adequacy of metabolic regulation and blood lipid levels i e all factors reportedly capable of accelerating or retarding the development of vascular complications

MATERIAL AND METHODS

The clinical material consisted of 221 randomly selected diabetics 104 women and 117 men who had a fasting blood glucose concentration above 120 mg/100 ml at the time of diagnosis and no signs of coexisting disease capable of explaining the abnormally high concentration The patients had had diabetes mellitus for on an average 10 2 years (range 0–39) 71 patients had had the condition for 15 years or more (Table I)

Laboratory studies Blood samples were obtained between 8 and 9 a m from fasting patients Blood and urine glucose were determined with the glucose oxidase method (19) and serum creatinine with a Technicon Auto-

Table I *Distribution of patients according to duration of disease presence or absence of overweight and age at onset*

	Juvenile onset type		Maturity onset type	
Age at onset (y)	0–14	15–29	30–44	45–
Total no	32	41	37	111
Duration (y)				
<1	0	8	5	26
1–9	0	12	17	54
10–19	16	10	8	30
20–39	16	11	7	1
Without overweight	32	39	24	49
With overweight (≥20 %)	0	2	13	62

Analyzer The method of Boy et al (8) was used for measuring plasma cholesterol and the plasma triglycerides were determined according to Kessler (18)

Before the patients were examined for the following fibrinolytic factors they had breakfast and those requiring insulin sulfonylurea or biguanide had their usual dose None were receiving any other drugs known to be capable of influencing haemostatic variables

Plasminogen activator activity of the vein walls The fibrinolytic activity of a biopsy specimen of a superficial dorsal vein of the hand was assessed histochemically by a modification of Pandolfi et al (23) of the histochemical of Todd (26) Normal range 6 0–10 0 arbitrary ts of possible 12 Median value 7 5

ntaneous fibrinolytic activity was measured by test ved euglobulin precipitate on fibrin plates (22) 153 age and sex matched healthy controls the mean spontaneous fibrinolytic activity was 34 4±S E 3.3 mm²

Fibrinolytic response to venous occlusion (*fibrinolytic capacity*) The local fibrinolytic activity developing during artificial venous occlusion of the arms was measured according to Robertson et al (25) In the age and sex matched controls the mean fibrinolytic activity after such occlusion was 313 8±S E 10 2 mm²

Fibrinogen Photometric method according to Nilsson and Olow (22) Normal mean 300±S E 6 mg/100 ml

Plasminogen was determined immunologically according to Ganrot and Nilehn (15) Normal range 70–130 %

α_2-macroglobulin Esterolytic method (14) Normal range 80–120 %

Urokinase inhibitors (inhibitors of plasminogen activation by urokinase) Clot method (24) Normal range 80–120 %

Fibrin/fibrinogen degradation products were determined immunologically (20) Normal range below 5 µg/ml

RESULTS

The *plasminogen activator activity of the vessel walls* was measured in 205 of the patients In 48 (23 4 %) the activity was abnormally low (less than 6 0 arbitrary units) Of those with a juvenile onset 17 4 % had an abnormally low activity and of those with maturity onset 26 5 % (Figs 1 and 2) The activator activity did not vary significantly with age sex and standing of diabetes but those with at least 20 % overweight had abnormally low levels significantly more often ($p<0\ 001$) than those below ideal weight

The *spontaneous fibrinolytic activity* An activity below 15 mm² of lysis was noted in 55 % of the diabetics many of whom had no measurable activity at all Of the controls 40 % had an activity below 15 mm² the difference from the incidence among the diabetics was significant ($p<0\ 01$) However the mean activity in the diabetics 30 5±S E 2 4 mm² did not differ significantly from that in the controls 34 4±3 3 mm² There was no significant difference between those with juvenile onset and those with maturity onset Neither did the activity vary with sex or age Patients with 20 % overweight or more had a significantly ($p<0\ 01$) lower activity than those of ideal weight or less

The mean *fibrinolytic response to venous occlusion* in those with juvenile onset of diabetes was 245 4±S E 12 3 and in those with maturity onset 240 8±S E 12 4 mm² of lysis both means being significantly lower ($p<0\ 001$) than the activity in the controls 313 8±S E 10 2 The fibrinolytic response did not vary with sex age or standing of diabetes A fibrinolytic response below 159 mm² of lysis which is the lower normal limit was observed in 66 subjects (30 %) compared with 11 expected 32 5 % of the patients with maturity onset belonged to this group and 24 6 % of those with juvenile onset (Figs 3 and 4) The fibrinolytic response decreased with increasing overweight 109 diabetics at or below ideal weight had a mean response of 278 2 mm² of lysis which was significantly higher ($p<0\ 001$) than the mean response of 188 3 mm² in the 77 with 20 % overweight or more

The *fibrinogen* level (mean 377±S E 8 mg/100 ml) was significantly higher than in the controls ($p<0\ 001$) There was no significant correlation between the fibrinogen level and sex age age at onset of diabetes or duration of diabetes

The *plasminogen* level did not differ from levels seen in controls the mean being 98 9 % with no significant variation with sex age or standing of diabetes

The mean level of *α_2-macroglobulin* 139±S E 4 % was significantly higher than in the controls

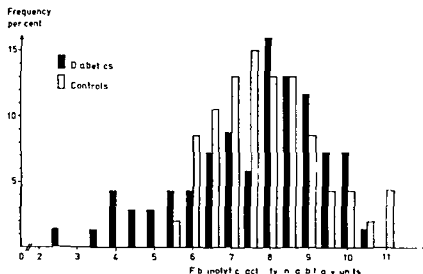


Fig 1 Frequency distribution of the fibrinolytic activity (histochemical method) of vein walls in subjects with juvenile onset of diabetes mellitus

($p<0.01$) with a marked sex difference the women having a mean of 152±S E 5% and the men 128±S E 5% The levels tended to be high early and late in life but substantially lower between 35 and 65 years of age There was a significant inverse correlation ($p<0.05$) between the α_2 macroglobulin level and the degree of overweight The mean levels were significantly higher ($p<0.001$) in those who had had diabetes for more than 20 years than in those who had had the disease for less than 10 years

The mean concentration of *urokinase inhibitors* was 124±S E 2% and was significantly higher than in the controls ($p<0.01$) There were no significant differences by sex or age degree of overweight or standing of diabetes

Fibrin/fibrinogen degradation products were found in small amounts (range 5–35 μg/ml median level 10 μg/ml) in 9 patients Most of these patients had microangiopathy The highest level was found in the only patient in this investigation who was in poor general condition and had uremia

Regulation of diabetes There were no significant differences in the fibrinolytic system between those who were properly regulated at the time of the investigation and those who were not (blood glucose level above 150 mg/100 ml and/or daily urine glucose output above 20 g) (Table II)

Blood pressure There were no significant correlations between the systolic or the diastolic BP and the fibrinolytic factors examined except that the fibrinolytic capacity was significantly higher

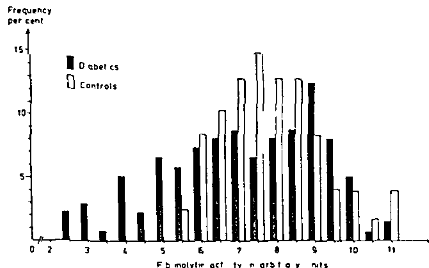


Fig 2 Frequency distribution of the fibrinolytic activity (histochemical method) of vein walls in subjects with maturity onset of diabetes mellitus

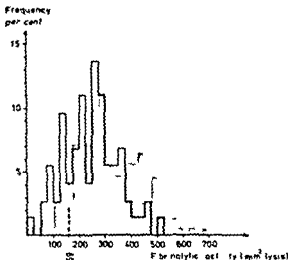


Fig. 3. Frequency distribution of fibrinolytic capacity in subjects with juvenile onset of diabetes mellitus. Shaded area = controls.

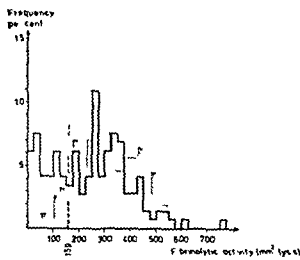


Fig. 4. Frequency distribution of fibrinolytic capacity in subjects with maturity onset of diabetes mellitus. Shaded area = controls.

($p<0.02$) in those with a diastolic pressure of at least 110 mmHg (328.3±S.E. 22.5) than in those with a lower pressure (95–109 mmHg 238.4±S.E. 21.3, below 95 mmHg 236.9±S.E. 10.9).

Plasma cholesterol and triglycerides. Hypercholesterolemia (>250 mg/100 ml) was found in ..% and hypertriglyceridemia (>2.2 mmol/l) in ... of the patients. Hypertriglyceridemia but not ...cholesterolemia was associated with obesity more often than among diabetics with normal plasma cholesterol and triglyceride levels. There were no significant correlations between the blood lipid levels and the fibrinolytic factors examined, but in hypercholesterolemia the mean spontaneous fibrinolytic activity and in hypertriglyceridemia the mean fibrinolytic capacity was only two thirds of the respective means in those without excess of these substances.

Smoking habits. 62% of the patients were non-smokers and many of these belonged to the maturity onset group and were more often overweight than smokers, whose weight was most often rather close to ideal. There was no significant difference between smokers and non-smokers concerning spontaneous fibrinolytic activity, fibrinolytic response to venous occlusion, fibrinolytic activity of the vessel walls, fibrinogen level and the concentrations of α-macroglobulin and urokinase inhibitors.

DISCUSSION

It has long been known that microangiopathy as well as macroangiopathy are common complications of diabetes, but it is not known why. Several investigators have suggested an imbalance between coagulation and fibrinolysis, with impaired removal of fibrin deposits and vascular lesions as a result. The spontaneous fibrinolytic activity of the blood is normally relatively low and available methods for measuring this activity are not very sensitive. However, it is now possible to assess the plasminogen activator activity in the tissue from which the activator is released and where it is most probably formed, the vessel wall. Fibrinolytic capacity, i.e.

Table II. *Regulation of diabetes*

	Spontaneous fibrinolytic activity (mm² lysis)	Fibrinolytic capacity (mm² lysis)	Urokinase inhibitors (%)
Good regulation	31.5±S.E. 3.7	249.1±S.E. 13.2	120±S.E. 3
Poor regulation	26.8±S.E. 4.0	235.5±S.E. 12.9	129±S.E. 4

the amount of this activator that is released to the blood from the vascular endothelium in response to a given stimulus provides a measure of the capacity of the release mechanism The determinations of this fibrinolytic capacity the fibrinolytic activity of the vessel wall and the spontaneous fibrinolytic activity of the blood are much more informative than data previously available

In this investigation we found these variables to be lower in 221 diabetics than in the controls There were however no significant differences between those with juvenile onset and those with maturity onset suggesting that the factors influencing the fibrinolytic system did not vary with the form of diabetes Though the fibrinolytic activity did not decrease with increasing duration of the diabetes the mean fibrinolytic capacity in patients with long standing diabetes was normal only in those who had not developed microangiopathy (4)

In a previous investigation (3) we reported a de creased fibrinolytic activity in diabetics with plethysmographically verified arterial insufficiency of the legs That finding together with the present observations suggest that a low fibrinolytic activity might be one of the factors contributing to vascular lesions in diabetes Overweight is frequently as sociated with angina pectoris and sudden death (16) and pulmonary embolism (9) We found that over weight correlated significantly not only with the spontaneous fibrinolytic activity and the fibrinolytic capacity but also with the activator activity of the vessel wall (1) However the co variation be tween fibrinolysis on the one hand and hyper cholesterolemia and hypertriglyceridemia on the other was not significant

No significant correlation was found between fibrinolysis and the adequacy or inadequacy of metabolic regulation of diabetes but good control at the time of the investigation did not necessarily imply that the regulation had always been good This uncertainty makes it difficult to draw any relevant conclusions We therefore believe that for the time being it is not possible to exclude a certain dependence of fibrinolysis on the adequacy of regu lation of the diabetes

Drugs that stimulate fibrinolysis such as phenformin and ethyloestrenol have been success fully tried by Fearnley et al (13) and Nilsson et al (21) in the prevention of thrombosis It is possible that such prophylaxis would decrease the risk of developing angiopathy in diabetes

ACKNOWLEDGEMENTS

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Phenformin and Ethyloestrenol in Recurrent Venous Thrombosis

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Abstract Altogether 75 patients 60 of whom had recurrent idiopathic deep venous thrombosis 10 recurrent superficial thrombophlebitis and 5 retinal venous thrombosis were given a combination of 100 mg phenformin and 8 mg ethyloestrenol a day for periods varying between 3 and 48 months (mean 16 2) Initially all the patients had an abnormally low plasminogen activator content in the walls of superficial veins and/or a decreased capacity to release plasminogen activator from the vein walls on venous occlusion of the arms The combination elicited a significant increase in the spontaneous fibrinolytic activity and in the local fibrinolytic activity during standardized venous occlusion of the arms (assayed on fibrin plates) after treatment for 3 months ($p<0\ 001$) These activities then remained normal throughout the observation period In 65 of the 75 patients studied the plasminogen activator level in the vein wall (histochemically assayed by the method of Pandolfi) was abnormally low initially The level had increased significantly after treatment for 3 months ($p<0\ 001$) and after 12 months there was a further significant increase ($p<0\ 01$) After treatment for 12 months all the patients except 2 were normal The patients in this material had had 2–20 episodes of venous thrombosis the most recent 5 years before the institution of treatment in spite of dicoumarol treatment During treatment 9 thrombotic episodes occurred in 5 of these patients the fibrinolytic activity of the vessel wall was not normal at the time of the recurrence Except in 1 patient who developed lactic acidosis the side-effects were not severe

Isacson and Nilsson (9) have shown that the majority of patients with recurrent idiopathic venous thrombosis have a decreased plasminogen activator content of the vein walls and/or a defective release of plasminogen activator from the vein walls Fearnley et al (4) reported that phenformin combined with ethyloestrenol stimulates the spontaneous fibrinolytic activity of the blood Isacson and Nilsson (8) later showed that when phenformin combined with ethyloestrenol was given to healthy volunteers it caused an increase in the plasminogen activator content of the vein walls It was therefore thought worthwhile to try this combination on patients with a history of recurrent venous thrombosis and an initially low fibrinolytic activity of the vessel walls

This paper reports 60 patients with recurrent idiopathic deep venous thrombosis 10 with recurrent superficial thrombophlebitis and 5 with retinal venous thrombosis All the patients presented an abnormally low plasminogen activator content of the walls of superficial veins and/or a decreased capacity to release plasminogen activator from the vein walls on venous occlusion of the arms They were treated with phenformin and ethyloestrenol for 3–48 months The various components of the fibrinolytic system and the fibrinolytic activity of the vessel wall were followed at regular intervals

MATERIAL AND METHODS

The clinical material consisted of

1) 60 patients (38 males 22 females) with phlebographically verified idiopathic venous thrombosis Their ages ranged from 17 to 70 years (mean 46 1) 18 of them were below 18 years During the last 5 years before the first examination the patients had had 2–20 thrombotic episodes several of which had been complicated by pulmonary embolism None of them had any demonstrable liver or renal disease or any other known coexisting disease or alcoholism On admission for the trial all the patients with recurrent deep venous thrombosis were receiving dicoumarol prophylactically

2) 10 patients (8 males 2 females) aged 26–59 years with recurrent histologically verified idiopathic thrombophlebitis with no demonstrable phlebographic changes Neither clinical nor laboratory studies had revealed any coexisting disease or complication.

3) 5 patients (4 males 1 female) aged 32–67 years with repeated retinal venous thrombosis These patients were otherwise apparently healthy

Table I *Duration of therapy*

Treatment period (mo)	Number of patients: Deep venous thrombosis	Superficial thrombophlebitis	Retinal venous thrombosis
3	13	3	1
6	5	–	–
12	15	2	–
24	15	4	1
36	8	–	2
48	4	1	1

The patients were seen at our laboratory on an average 3 months after the end of their last thrombotic episode. Each patient was examined on 2 consecutive days and 70 were reexamined just before the beginning of treatment 3–6 months later.

Only patients with an abnormally low plasminogen activator content in the walls of superficial veins and/or an abnormally low local response of the fibrinolytic activity to venous occlusion of the arms at repeated investigations were accepted. The patients were given phenformin timed release capsules (Dibein Retard®) 50 mg twice a day plus ethyloestrenol (Orgabolin®) 4 mg twice a day. The duration of the therapy is given in Table I. The patients were reexamined after 3–6, 12, 24 and 36 months and a few of them also after 48 months.

Dicoumarol therapy was given for the first 3 months of therapy but no other antithrombotic drugs.

The patients were carefully followed up for recurrences of thrombosis and for side-effects. The concentration of serum creatinine was measured at 3–6-month intervals.

Laboratory methods

The following determinations were made: platelet adhesiveness in whole blood, fibrinogen, plasminogen (immunochemical method), inhibitors of plasminogen activation by urokinase, α_2-macroglobulin and fibrinolytic activity of resuspended euglobulin precipitate of plasma on unheated fibrin plates. The procedures have been described elsewhere (3, 6, 7, 10, 13).

Venous occlusion. This was produced by placing a sphygmomanometer cuff around each upper arm and inflating it to a pressure between the systolic and diastolic BP for 20 min. Blood samples for determination of the fibrinolytic activity of resuspended euglobulin precipitate on fibrin plates were obtained before application and just

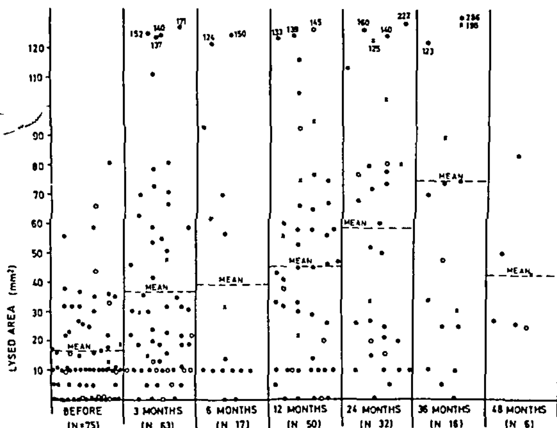


Fig. 1. Spontaneous fibrinolytic activity—resuspended euglobulin precipitate of plasma on unheated fibrin plates—during the course of treatment. N = no. of patients assayed on each occasion. ● = deep venous thrombosis (DVT). O = superficial thrombophlebitis (ST). × = retinal venous thrombosis (RVT).

LYSED AREA (mm²)
LOWER NORMAL LIMIT
MEAN
BEFORE (N 75) | 3 MONTHS (N=64) | 6 MONTHS (N 17) | 12 MONTHS (N 50) | 24 MONTHS (N 35) | 36 MONTHS (N 16) | 48 MONTHS (N=6)

Fig 2 Local fibrinolytic activity during venous occlusion of the arms throughout the treatment The mean fibrinolytic activity of resuspended euglobulin precipitate on unheated fibrin plates of both arms is given Symbols as Fig 1

before deflation of the cuff The mean increase in the fibrinolytic activity in the samples from each arm was taken as a measure of the fibrinolytic capacity

The control material consisted of 118 apparently healthy volunteers aged 18–50 The 5 percentile for the arms is 158 mm² (95% confidence interval 88–169 mm²) (14)

Assay of plasminogen activator content in superficial arm veins A segment of a hand vein was excised under local anaesthesia (0 5% Carbocain) The biopsy specimens were examined by Pandolfi s modification of Todd s fibrinolysis autography technique (11 12) The activity was expressed in arbitrary units according to Pandolfi et al (11) The median value found at our laboratory in 70 biopsy specimens of hand veins from healthy volunteers is 7.5 arbitrary units (range 6–10)

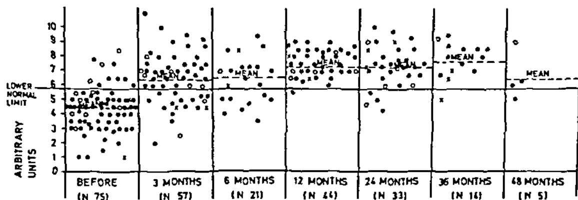


Fig 3 Plasminogen activator content in the vein walls expressed in arbitrary units during treatment Symbols as in Fig 1

Table II *Mean values for platelet adhesiveness and the various components of the fibrinolytic system before and during treatment*

Treatment period (mo)	Platelet adhesiveness (%)	Fibrinogen (g/100 ml)	Plasminogen (%)	α_2-macroglobulin (%)	Inhibitors of plasminogen activation (%)
Before treatment	25	0 37	106	105	114
3	23	0 31*	125	94	108
6	25	0 33	118	86	126
12	24	0 31	120	95	111
24	24	0 33	130	82	120
36	22	0 30	120	96	128
48	–	0 33	130	116	129

* $p<0\ 001$

RESULTS

Fig 1 gives the spontaneous fibrinolytic activity of the circulating blood as determined in resuspended euglobulin precipitate on unheated fibrin plates The spontaneous fibrinolytic activity was significantly increased after 3 months therapy ($p<0\ 001$) It then persisted at about the same level throughout therapy

The local fibrinolytic activity during standardized venous occlusion of the arms during the treatment is given in Fig 2 The combination caused a ficant increase in the fibrinolytic activity 001) measured after 3 and 6 months treat The mean activity then remained normal throughout the observation period without further significant increase Before treatment the local response of the fibrinolytic activity to venous occlusion of the arms was abnormally weak—i e below 158 mm² which is the lower limit of our normal range—in 34 patients After 3 months treatment the activity was low in only 8 patients After 12 months treatment it was markedly low in only one patient borderline values (120–150 mm²) were recorded in 5 patients No significant increase occurred in any of the 41 patients who had normal fibrinolytic activity on venous occlusion before the treatment

Altogether 65 of the patients had an abnormally low plasminogen activator content in the vein walls before treatment (Fig 3) The mean level of plasminogen activator rose significantly after treatment for 3 months ($p<0\ 001$) After treatment for 12 months a further significant increase occurred ($p<0\ 01$) but the level then remained about the same throughout the treatment period After treatment for 3 months 20 patients still had plasminogen activator levels below 6 arbitrary units which is the lower normal limit in our control material After 12 months treatment only 3 of the 44 patients tested had low values

Three patients did not show any improvement of the vessel wall fibrinolytic activity after treatment for 6 months The treatment was therefore stopped In 2 patients the fibrinolytic activity of the vessel wall was still low after treatment for 12 months

Fibrinogen decreased during the therapy while plasminogen increased No significant change occurred in platelet adhesiveness in the content of inhibitors of plasminogen activation and α_2-macroglobulin (Table II)

The number of recurrences during treatment is given in Table III Altogether 9 thrombotic episodes occurred but in 5 of the patients the fibrinolytic activity of the vessel wall was not normal at the time of recurrence

No local recurrences were observed in any of the 5 patients who had had venous thrombosis of the retinae A substantial improvement was noted in 9 of the 10 cases of thrombophlebitis

The following side-effects were observed Of 14 women of fertile age 4 become amenorrhoic One woman 50 years old reported symptoms of depression and treatment was withdrawn After 3 years treatment 1 man aged 33 developed lactic acidosis for which no other explanation could be found

DISCUSSION

The present trial was undertaken to evaluate the effect of combined phenformin and ethyloestrenol for longer periods on the fibrinolytic activity of the blood and vessel walls in patients with thrombosis and with an initially low content of plasminogen

Table III *Recurrences in the whole material during treatment*

Treatment period (mo)	No of pats	No of recurrences	Time of recurrence after beginning of treatment (mo)	Fibrinolytic activity in vessel wall at time of recurrence	
				Low	Normal
48	6	0			
36	10	2			
			12		+
			5	+	
24	20	4			
			3	+	
			24	+	
			5		+
			24		+
12	17	2			
			5		+
			3	+	
6	5	0			
3	17	1	1/2	+	

activator in the vein wall and/or a defective release of plasminogen activator from the vein walls

Like others (1 2 5) we found that this combination significantly increased the spontaneous fibrinolytic activity of the blood as long as treatment was continued But the spontaneous fibrinolytic activity is normally so low that it is difficult to draw any conclusions in a given case We therefore measured the local fibrinolytic activity during standardized venous occlusion of the arms a method we have found to be much more sensitive for measuring the fibrinolytic capacity of a given person (14) Within 3 months the combination caused a significant increase in the fibrinolytic activity as measured with this method No further significant increase occurred in the fibrinolytic activity during treatment Before treatment 34 of the 75 patients had a weak release of plasminogen activator on venous occlusion After treatment for 3 months the activity increased to normal levels in most of these patients It is of interest that no significant increase occurred in any of the 41 patients who had normal fibrinolytic activity on venous occlusion before treatment

In 65 of the 75 patients the plasminogen activator level in the vein was abnormally low before treatment The combination caused a significant rise after treatment for 3 months However in 20 patients the activator level had by that time not become normal After treatment for 12 months all the patients except 2 were normal The mean rise of activator level between the treatment for 3 and 12 months was significant

An urgent question is how long phenformin and ethyloestrenol must be given before the combination has its full effect on fibrinolytic activity Fearnley et al (4) claimed that the full effect was not obtained until after some 8–12 weeks treatment In a pilot study we found that in patients with an initially low content of plasminogen activator the level did not become normal after 1 month s treatment In the present investigation the fibrinolytic activity in the vessel walls was therefore not checked until after 3 months treatment It is clear from our investigation that this interval is not long enough for the activator content to become normal in all patients After 12 months treatment however the fibrinolytic activity become normal in all patients except 2 who were obviously resistant to treatment On the other hand the fibrinolytic activity after venous occlusion often became normal after 3 months. It therefore appears that the synthesis of the plasminogen activator in the vein wall does not became normal as soon as the release mechanism

Fearnley et al (4) pointed out that the fibrinolytic effect of ethyloestrenol disappeared after 3–4 weeks treatment despite continued increase in dosage Resistance to the fibrinolytic effect of phenformin on the other hand takes 3–4 months to

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Urinary Excretion of Glycosaminoglycans in Malignant Diseases of the Haemopoietic and Lymphatic Tissues

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Abstract A study has been made of the urinary excretion of glycosaminoglycans (GAG) in 50 patients with malignancies including 6 patients with acute myeloid leukaemia (AML) 11 with chronic myeloid leukaemia (CML) 10 with chronic lymphatic leukaemia (CLL) 10 with multiple myeloma (MM) 7 with Hodgkin s disease and 6 with mycosis fungoides (MF) The total urinary GAG were isolated by precipitation with cetyltrimethyl ammoniumbromide (CTAB) and assayed in terms of their hexuronic acid content A statistically highly significant increase in the excretion of total GAG was observed in all the disorders studied except Hodgkin s disease the highest values being seen in myeloid leukaemia (ML) Constant amounts of non-dialysable urinary GAG were electrophoresed in 0 5 M lithium acetate on cellulose acetate strips and stained with alcian blue The densitometric tracings derived from the electrophoresis strips were analysed with a Du Pont Curve Resolver The electrophoretic data suggested the existence of a qualitative deviation in GAG excretion in CLL and in MF in that patients with these diseases excreted on an average larger than normal amounts of slowly migrating GAG fractions Pooled crude urinary GAG material from patients with CLL, MF AML and CML and from control subjects was further purified and subjected to analytical studies These indicated that a similar qualitative urinary GAG distribution exists in ML and in controls whereas the urinary GAG in CLL and MF patients contained relatively more dermatan sulphate (DS in terms of iduronate) than those of the controls

Numerous studies have established the occurrence of a grossly elevated qualitatively abnormal urinary excretion of GAG in genetic mucopolysaccharidoses (11) However little work has been done on the excretion of GAG in neoplastic diseases of connective or related tissues Rich and Myers (24) have investigated GAG excretion in 29 patients suffering from disseminated malignant diseases The average daily GAG hexuronic acid excretion was found to be 15 9 mg in 7 patients with AML 15 5 mg in 2 patients with CML and 11 7 mg in 3 patients with MM the range of normal excretion being 2 8–7 5 mg Neoplasias originating in mesenchymal tissues were associated with a higher excretion of GAG than were carcinomas A chemical analysis of the GAG collected from the urine of two patients with AML did not disclose any significant difference from that of healthy subjects

A previous study (14) designed to detect pathological GAG excretion in patients with various connective tissue disorders included patients suffering from malignancies of the haemopoietic or lymphatic tissues The urinary GAG excretion in the latter patients has been subjected to additional studies the results of which are reported below along with data from the initial study

MATERIAL AND METHODS

Urine was collected during 24 to 48 hour periods from 50 patients with the following diseases 6 cases of AML 11 of CML 10 of CLL 10 of MM 7 of Hodgkin s disease and 6 cases of MF Most of the patients received steroid or cytostatic treatment during the course of the study Control samples (from 64 persons) were provided by hospital staff or by patients undergoing hospital treatment for minor non-metabolic or non infectious disease The preservative utilized during urine collection was 1 ml of 2.5% merthiolate A creatinine content of the specimen of less than 0 8 g was considered to be indicative of urine losses during collection Samples of this nature were included in qualitative studies only

Quantitative determination of total urinary GAG excretion Urinary GAG were isolated from 200–2 000 ml urine aliquots by application of the method of Teller et al (26)

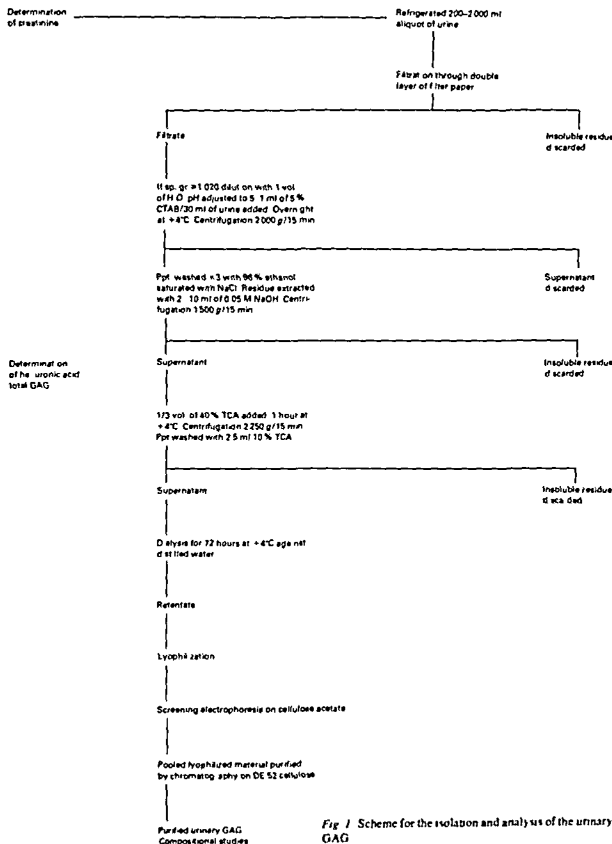


Fig 1 Scheme for the isolation and analysis of the urinary GAG

scaled up for the large samples analysed Hexuronate was assayed by the carbazole (9) and orcinol (1) procedures Calculations were also made of the hexuronate (carbazole) excretion in relation to body surface and creatinine excretion The average recovery of added chondroitin-4 sulphate (CS-4) was 91% and that of added hyaluronate (HA) 96% The precision of the method was evaluated by assaying the GAG content of twelve 200 ml aliquots of pooled urine The coefficient of variation obtained with the carbazole procedure was 1 6% and with the orcinol procedure 4 1% The influence of initial sample volume upon the results was negligible Mean values were calculated when analyses were made of two urine specimens from one and the same subject The results are reported as mean ±S D The differences between means were evaluated statistically with Student s *t* test

Electrophoretic screening study of the urinary GAG Prior to electrophoresis the dissolved CTAB GAG precipitate was deproteinized with trichloroacetic acid (TCA) dialysed and lyophilized (Fig 1) Aqueous solutions containing 0 9 or 1 8 μg/μl hexuronate were prepared A volume of 1 μl of the sample was applied to Oxoid (Courtaulds Ltd Coventry UK) cellulose acetate strips 5×36 cm in size along with 1 μl of a mixed HA CS-4 and heparin standard containing 1 μg of each component Electrophoresis was effected in an LKB apparatus (LKB Produkter AB Stockholm Sweden) The running time was 5 hours the applied potential gradient about 3V/cm and the medium was 0 5 M lithium acetate ionic strength 0 50 and pH 7 5 Staining was done with alcian blue (13) Photoscanning of the dried electrophoretic strips was performed with a Chromoscan (Joyce Loebl and Co Ltd Team Valley Gateshead UK) Most of the densitometric curves were analysed with a Du Pont 310 Curve Resolver (E I Du Pont de Nemours & Co Inc Wilmington Delaware USA) a special purpose analogue computer designed for the rapid resolution of overlapping peaks in experimental curves (3) The computer was operated as follows 1) The synthetic curve was constructed by beginning from the main peaks in the densitometric tracing 2) Use was made of the minimal number of synthetic components of Gaussian configuration necessary for synthesis of the desired curve 3) The instrument was operated without the employment of skewed peaks The electrophoretic components thus obtained were treated by plotting their mobilities (relative to standard CS-4=m_{CS-4}) against their percentage contributions to the sum curve of a densitometric tracing The data thus derived from the same group of subjects under study were subjected to linear regression analysis The slopes of the regression lines in the disease groups were compared with those of the control group

Analytical studies of pooled urinary GAG fractions Lyophilized material (Fig 1) from controls and from patients with AML CML CLL and MF was pooled groupwise and purified by chromatography at room temperature on Whatman DE 52 (W & R Balston Ltd Springfield Mill Maidstone Kent UK) anion exchange columns 0 9×20 cm in size which had been precycled with 2 0 M sodium chloride and 0 025 M phosphate buffer in 0 05 M sodium chloride The column was eluted with a linear gradient of sodium chloride produced by a Varigrad gradient mixer (Boskamp Geratebau 5304 Hersel bei Bonn FRG) at a flow rate of 20 ml/hour The chloride concentration of the effluent was measured by titration with silver nitrate and the hexuronate concentration was assayed with a borate–carbazole procedure (7) The contents of hexuronate-containing tubes (3–6 mg hexuronate) were combined dialysed against water and rechromatographed on DE 52 columns which had been precycled with water only and from which the GAG were eluted and assayed as described above

Because of a scarcity of material which would have prevented adequate analytical characterization of the minor fractions and since comparison between the disease groups and the controls was the chief aim of the experiment all hexuronate containing fractions of a run were then pooled dialysed against water lyophilized weighed dissolved in water and subjected to the following analyses hexosamine (12 20) quantitative determination of glucosamine and galactosamine (17) hexuronate (7) iduronate (6) hexose (22) sulphoaminohexose (21) sulphate (27) nitrogen (Kjeldahl) sialic acid (28) fucose (10) and deoxyribose (8) Samples containing 2 5 μg hexuronate/μl were electrophoresed on cellulose acetate membranes in pyridine formic acid pH 3 0 (18) and in 0 1 M barium acetate (30) Samples were digested with testicular hyaluronidase (I H 2001 Sigma Chemical Co St Louis Missouri USA) as follows 12 5 mg of enzyme in citric acid–phosphate buffer pH 6 0 was incubated with 1 ml of sample containing about 100 μg hexuronate at 37°C and dialysed against the buffer for 24 hours and then against water for 24 hours lyophilized and electrophoresed in pyridine formic acid together with an identically treated sample without hyaluronidase Results were evaluated by visual inspection In order to check the procedure samples of standard HA and CS-4 were also digested

RESULTS

Fig 2 shows the normal range of total GAG excretion along with the individual patient excretion values which display considerable variation Excretion was elevated most consistently in ML exceeding the normal range in all cases of AML and in 8 of 11 cases (73%) of CML An extremely high GAG excretion (69 3 mg/24 h) was observed in one patient with AML In the other diseases the excretion showed a considerable overlap into the normal range Data on the excretion of total GAG are given in Table I An increased excretion highly significant statistically is discernible in all the diseases studied except for Hodgkin s disease

Fig 3 illustrates the results of the electrophoretic screening study The regression lines obtained by analyses of the electrophoretic data from the different patient groups are compared with the regression line of the control group (based on the data from 47

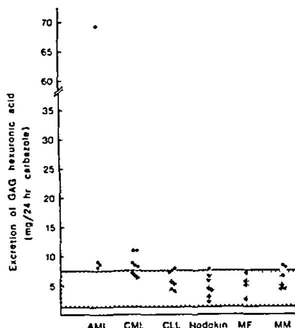


Fig 2 Excretion of total $\bar{G}$AG (expressed as carbazole hexuronic acid) The shaded area represents the range (mean±2 S D) of urinary GAG excretion in 64 adult con trols

subjects) It is evident that the slopes of the regres lines in CML MM and Hodgkin s disease are similar to the control slope The slopes for and to a greater extent, MF and CLL are than the control slope indicating that more GAG fractions with low electrophoretic mobilities are excreted in these diseases than in the controls On the basis of these results material from patients with AML MF and CLL was pooled groupwise for further studies In view of the markedly elevated total GAG excretion in CML pooled material from CML patients was also analysed further

Fig 4 shows the elution patterns of the pooled urinary GAG samples when rechromatographed on DE 52 columns The main GAG peak was eluted with about 0 25 M sodium chloride in the controls AML and CML patients with about 0 30 M in CLL patients and with 0 20 M in MF patients Consider able variations in elution patterns are discernible between the groups Since all hexuronate-con taining material was pooled before the analytical studies the chromatographic runs represented steps of purification only by which proteinaceous material with an anionic charge lower than the GAG was removed from the samples

The results of the analytical studies are shown in Table II The high contents of nitrogen and hexose and the presence of sialic acid and fucose in the fractions indicate contamination with fragments of uromucoid the only glycoprotein in urine known to precipitate with CTAB in significant amounts (4) The fractions do not contain deoxyribonucleic acid as evidenced by the absence of deoxyribose Galactosamine is the predominant hexosamine Hexuronate is present in approximately equimolar amounts with hexosamine Sulphate values are low and correlate to the chromatographic elution pat terns Iduronate values are higher than expected especially in CLL and MF

Table III shows the distribution of GAG in the samples as calculated from the data in Table II It is evident that the ML patients and controls resemble each other closely in GAG distribution In the malignant lymphomas CLL and MF the urinary

Table I *Urinary glycosaminoglycans in haemopoietic and lymphatic tissue malignancies*

		Hexuronic acid (carbazole)		Hexuronic acid		
				Carbazole		Carbazole/ orcinol
	N	mg/24 h	mg/24 h/m²	creatinine (mg/g)	Orcinol (mg/24 h)	ratio
AML	6	23 14±23 91***	13 60±16 00* *	20 20±22 74 *	17 54±17 61***	1 33±0 23
CML	11	11 99± 7 95 **	6 85± 4 40 **	10 70± 6 26 **	8 05± 3 65***	1 31±0 17
CLL	10	6 72± 2 21**	3 88± 1 22 *	6 12± 2 52*	5 00± 1 55 *	1 43±0 32 *
Hodgkin	7	4 79± 2 02	2 97±0 71	4 57± 1 31	4 27± 1 39	1 12±0 31
MF	6	13 56±13 02	7 11± 6 75 *	5 88± 3 22 **	10 60± 7 00	1 03±0 26
MM	10	10 40± 8 09	6 04± 4 63 *	8 60± 6 87	8 78± 6 76	1 20±0 20
Controls	64	4 31± 1 62	2 42± 0 91	3 36± 1 21	4 31± 1 74	1 05±0 26

The significance of the differences as compared with the controls $0.01<p<0.05$ (almost significant) * $0.001<p<0.01$ (significant) ** $p<0.001$ (highly significant)

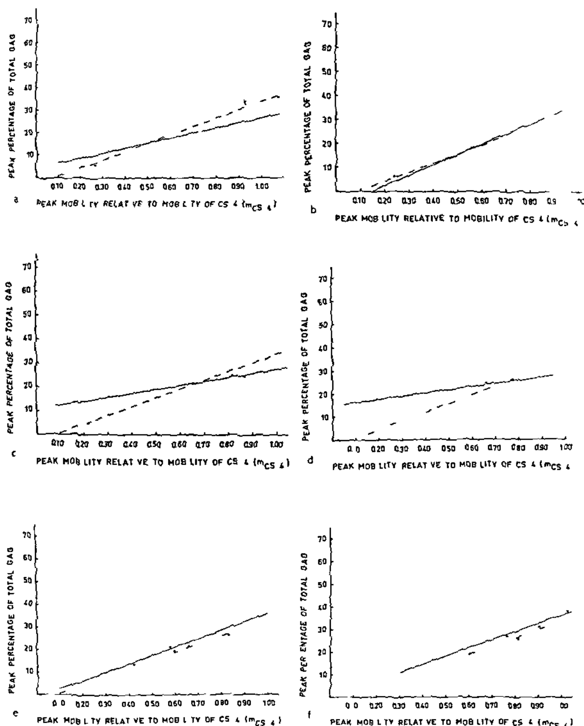


Fig 3 Linear regression analysis of the data derived from the electrophoreses of urinary GAG in 6 cases of AML (*a* line equation y=22 1x+4 438) 10 cases of CML (*b* line equation y=42 9x 6 155) 9 cases of CML (*c* line equation y 17 3x+10 43) 4 cases of MF (*d* line equation y=13 8x+14 63) 10 cases of MM (*e* line equation y=37 1x 0 951) and in 7 cases of Hodgkin s disease (*f* line equation y 35 9x+0 374) =the regression line (equation y=37 1x 3 273) derived from the electrophoretic data of 47 controls

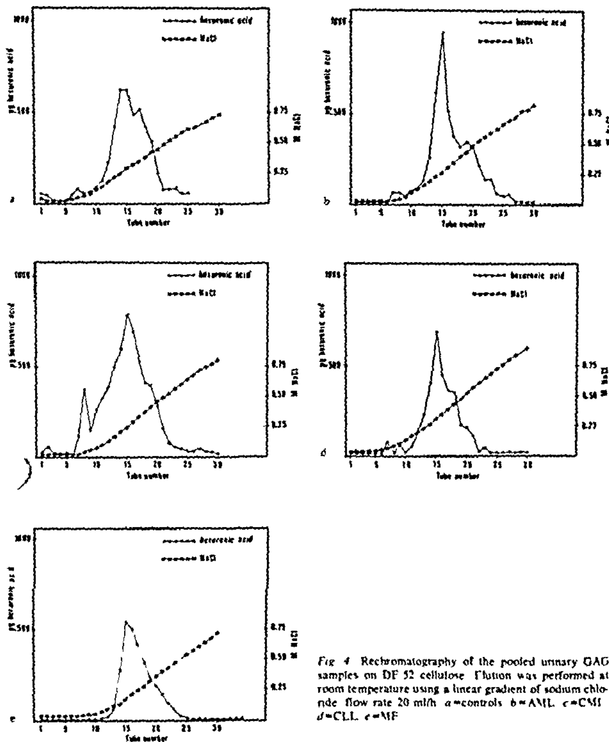


Fig 4 Rechromatography of the pooled urinary GAG samples on DE 52 cellulose. Elution was performed at room temperature using a linear gradient of sodium chloride flow rate 20 ml/h *a*=controls *b*=AML *c*=CML *d*=CLL *e*=MF

GAG contain relatively more DS and less CS and heparan sulphate (HS) than in the controls. Possible variations in the relative amounts of keratan sulphate (KS) of the different groups are difficult to evaluate because of the presence of glycoprotein in the KS fraction

DISCUSSION

It is thought that the urinary excretion of GAG reflects the metabolic state of connective tissue proteoglycans although the biochemical mechanisms involved are not fully understood. An increased urinary excretion may be attributable to alterations

Table II *Properties of the purified urinary glycos aminoglycan fractions*

The analytical data are molar ratios with hexosamine as signed the value 1 00

	Con trols	AML	CML	CLL	MF
Hexosamine	1 00	1 00	1 00	1 00	1 00
Glucosamine	0 38	0 28	0 39	0 36	0 32
Galactosamine	0 62	0 72	0 61	0 64	0 68
Hexuronate	0 95	1 06	1 27	0 86	0 99
Iduronate	0 15	0 17	0 05	0 31	0 27
Hexose	0 47	0 58	0 55	0 62	0 50
Sulphoamino-hexose	0 08	0 05	0 09	0 03	0 03
Sulphate	0 21	0 17	0 19	0 24	0 12
Nitrogen	3 57	4 82	2 33	3 88	4 09
Sialic acid	0 07	0 09	0 06	0 15	0 06
Fucose	0 07	0 08	0 09	0 09	0 07
Deoxyribose	–	–	–	–	–
Hyaluronidase re sistant GAG (%)	~15	~10	~10	~25	sl [a]
HS fraction by elec trophoresis[b]	++	+	++	++	+

[a] Sample lost [b] Heparan sulphate by electrophoresis in barium acetate

in body content turnover or the metabolic fate of a proteoglycan As with proteins the amount of GAG excreted is extremely minute in relation to the total quantity undergoing metabolization The pathways of proteoglycan catabolism are largely unknown although it is believed that lysosomal enzymes play an important role in this process

Many reports (2 15 25) describing the GAG frac tions of human plasma or serum point towards similarities between these and the urinary GAG a

Table III *Distribution of glycosaminoglycans in the purified fractions as calculated from the data in Table II (percentages of total hexosamine)*

	CS[a]	HS[b]	DS	KS+residual uromucoid[d]
Controls	47	16	15	22
AML	55	10	17	18
CML	56	18	5	21
CLL	33	6	31	30
MF	41	6	27	26

[a] Total galactosamine—dermatan sulphate galactosamine=chondroitin sulphate galactosamine
[b] Based on sulphoaminohexose content assuming an average 50% N-sulphation of heparan sulphate
[c] Based on iduronate content assuming an equimolar iduronate/galactosamine ratio
[d] Calculated as total glucosamine—heparan sulphate glucosamine=keratan sulphate+uromucoid glucosamine

fact which suggests that the latter derive ma The renal handling of plasma GAG glomerular filtration and partial tubu tion which covers about 90% of the CS through the glomeruli (31) It is thought t GAG do not derive from the lower (19) but some GAG may have a renal or

The findings reported here of a mar vated GAG excretion in MM AML ar firm and extend earlier observations (24 mal excretion noted in Hodgkin s disease iance with one report (24) but agrees with (16) The present study also demonstrates vated GAG excretion in CML and in MF Pre bly most of the excess GAG excreted in ML from leukaemia cells which are capable thesizing substantial amounts of GAG (23)

The excess GAG excreted in the other nancies studied may also be products of the plastic cells Alternatively the increased u GAG excretion may be due to a change in t teoglycan metabolism of normal connective induced by the neoplastic cells Human l cytes in culture produce only small quantities o (5) The data presented here suggesting incr excretion of DS in CLL and MF might be sec ary to the common skin involvement in these d eases DS being the main GAG of skin along with HA

ACKNOWLEDGEMENTS

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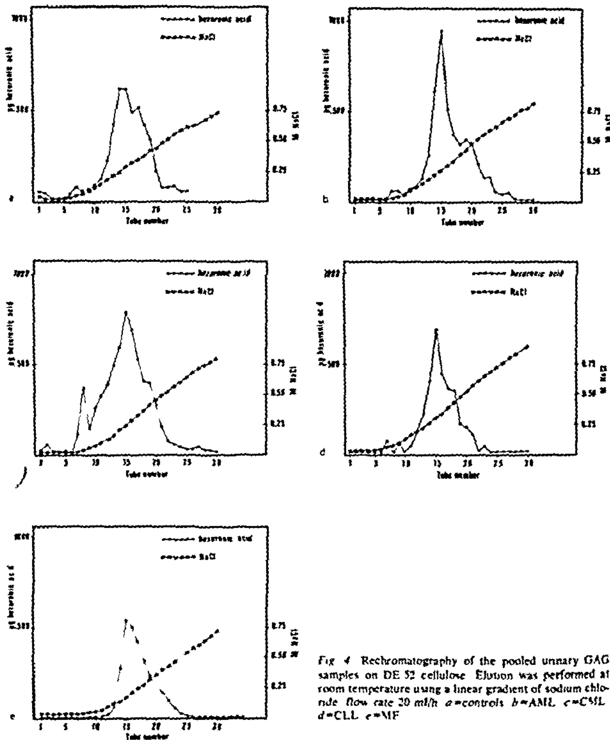


Fig. 4. Rechromatography of the pooled urinary GAG samples on DE 52 cellulose. Elution was performed at room temperature using a linear gradient of sodium chloride, flow rate 20 ml/h. *a*=controls, *b*=AML, *c*=CML, *d*=CLL, *e*=MF

GAG contain relatively more DS and less CS and heparan sulphate (HS) than in the controls. Possible variations in the relative amounts of keratan sulphate (KS) of the different groups are difficult to evaluate because of the presence of glycoprotein in the KS fraction.

DISCUSSION

It is thought that the urinary excretion of GAG reflects the metabolic state of connective tissue proteoglycans, although the biochemical mechanisms involved are not fully understood. An increased urinary excretion may be attributable to alterations

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Effects of Medium Chain Triglycerides on Portal and Arterial Levels of Insulin, FFA and Gl
in Patients with Pancreatic Disease

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ABSTRACT Trioctanoin has been given orally to six patients with pancreatic disease and the effects on the portal and arterial levels of insulin free fatty acids (FFA) and glucose have been investigated The serum levels of insulin and FFA rose significantly in portal blood but not in arterial blood after trioctanoin The glucose level in portal as well as in arterial blood remained unaffected by the fat ingestion The implications of these findings are discussed

Previous deductions regarding the effect of med um chain triglycerides (MCT) on insulin secretion have been based on in vitro experiments (9) or on insu l n stud es in the peripheral blood of man Some investigators have found no change in the basal peripheral level of human insulin after intra duodenal infusion of octanoic acid (6) Others have been able to show a significant increase in peripheral venous insulin after ingest on of MCT in patients with portal systemic commun cat ons and c rrhos s of the liver In healthy subjects and c rrhot ics without portal systemic communications no change has been found (7)

MCT contain approximately 80% octano c acid which after intestinal absorption is transported to the liver via the portal vein (7) Small amounts of MCT are found in ordinary diets consumed by man (4) Various ideas have been put forward regard ng the mechanism by which MCT may induce release of insul n One is that octano c acid m ght exert a direct stimulatory effect on the pancreat c β-cell (6 9) Another suggest on is that the MCT may cause a release of some factor from the intestinal mucosa wh ch in turn might induce insul n release (5 6)

In the present investigation insu n h termined both in portal and in arterial b and after ingestion of trioctano n in a g tients with pancreatic disease but w t liver impairment Moreover the portal levels of free fatty acids and gluc stud ed simultaneously in an attempt mechanism by which MCT may stir le lease of insul n in this group of patients

MATERIAL

In order to explore the portal system umb lical ve n n nulat on followed by portographic exam na on wa performed n patients adm tted to the Surgical Depart ment Sodersjukhuset because of overt or suspected hepatic or pancreat c d sease As pat ents w th severe hepat c mpa rment were excluded from th s study only those w th pancreatic d seases were invest gated Five (nos 1 5) were men (aged 20–49 years) with pancreat t s and one (no 6) was a woman (52 years of age) w th carcinoma of the pancreas At the t me of the invest ga t on symptoms and s gns typical of pancreat t s had sub s ded A normal hosp tal d et had been g ven to all the pat ents for at least three days prior to the experiments All were non-obese accord ng to the criteria for des rable we ghts g ven by the Metropolitan L fe Insurance Com pany in Documenta Geigy 1960 None had glucosuria or a fam ly h story of d abetes and none had portal system c commun cat ons at portograph c exam nat on Further data are given in Table I

METHODS

Blood glucose was determined enzymat cally by a glucose ox dase method us ng commerc al reagents (Glox Kab Stockholm Sweden) The error of the method calculated from 15 dupl cate fasting determ nat ons accord ng to the formula $\sqrt{\Sigma d^2/2n}$ was +1.2 mg/100 ml

Table I *Laboratory data and diagnoses*

GOT=glutamic-oxaloacetic transaminase GPT=glutamic pyruvic transaminase S A=coagulation activity as measured by Simplastin A (% of control)

Case no	Sex	Bilirubin (mg/100 ml)	GOT (U/ml)	GPT (U/ml)	S A (%)	Port pressure (mmH_2O)	Diagnosis
1	♂	0 6	26	28	130	190	Relapsing acute pancreatitis
2	♂	0 6	22	10	130	80	Acute pancreatitis
3	♂	0 6	14	8	88	150	Relapsing acute pancreatitis
4	♂	0 7	16	18	88	100	Acute pancreatitis
5	♂	0 8	16	13	88	120	Relapsing acute pancreatitis
6	♀	0 3	24	12	130	120	Carcinoma of the pancreas
Normal values		<1 2	<40	<35	70–130	<150	

Immunoreactive insulin in serum (IRI) was assayed by a double antibody procedure essentially as described by Soeldner and Slone (10) Porcine insulin (10×crystallized) was used for immunization All insulin concentrations were determined by reference to a standard of 2×crystallized human insulin (obtained from Dr J Schlichtkrull Novo Research Institute Copenhagen Denmark) The error of the method calculated from 20 duplicate determinations according to the above formula was ±0.8 μU/ml at insulin concentrations between 6 and 15 μU/ml and ± 1 3 μU/ml at concentrations between 16 and 50 μU/ml

Free fatty acids in serum (FFA) were assayed according to a colorimetric method as described by Duncombe (1) A commercial kit was used (Biochemica Test Combination Boehringer Mannheim West Germany) In this laboratory the error of the method calculated from 11 duplicate fasting determinations was ±0 04 mEq/1 000 ml

Portal vein catheterization was performed for diagnostic

Table II *Glucose FFA and IRI in portal and arterial blood before and after trioctanoin*

Basal=mean of two samples obtained 5 and 2 min before ingestion of trioctanoin

	Portal blood					Arterial blood				
	Basal	30	60	90	120′	Basal	30	60	90	120
(mg/100 ml)										
1	72	76	78	73	73	80	82	90	93	90
2	86	86	86	80	74	86	91	84	82	76
3	88	91	89	88	85	94	93	92	96	94
4	82	87	82	83	85	88	89	83	83	87
5	65	55	63	65	–	56	48	56	61	–
6	117	117	117	117	114	116	114	111	108	102
Paired diff		N S	N S	N S	N S		N S	N S	N S	N S
FFA (mEq/1 000 ml)										
1	0 54	0 72	0 68	0 44	0 48	0 44	0 45	0 36	0 18	0 17
2	0 71	1.54	1 78	1 60	1 57	0 77	0 83	0 74	0 72	0 68
3	0.57	0 84	0 79	0 92	1 03	0 32	0 33	0 31	0 27	0 32
4	0 42	0 62	0 51	0 45	0 46	0 30	0 29	0 34	0 31	0 35
5	0 70	1 08	1 05	2 12	–	0 57	0 78	0 56	0 41	–
6	1 10	2 25	2 23	1 64	1 57	0 96	1 08	0 68	0 57	0 50
Paired diff		<0 05	<0 05	N S	N S		N.S	N S	N S	N S
Insulin (μU/ml)										
1	11	14	15	22	18	3	4	5	6	4
2	6	10	11	12	8	1	4	1	2	2
3	8	21	16	13	12	8	7	7	12	11
4	14	19	29	18	16	13	13	11	12	14
5	16	20	34	34	–	3	5	6	5	–
6	13	15	22	18	13	11	6	8	6	5
Paired diff.		<0 05	<0 01	<0 02	N S		N S	N S	N S	N S

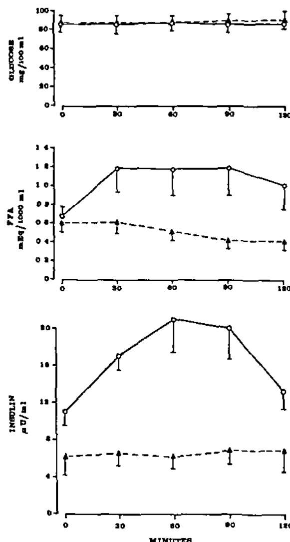


Fig 1 Glucose FFA and IRI in the portal (O—O) and arterial blood (▲--▲) before and after ingestion of trioctanoin in six patients with pancreatic disease (mean±S E M)

purposes in each patient (portal venography and manometry) A transumbilical technique was used (2 8 11) The tip of the catheter was placed in the common portal vein under fluoroscopic control and a slow drip of heparinized saline kept the catheter patent The catheter was left for a few days without any essential discomfort for the patients who were able to move freely in the ward and eat regular hospital meals 2–4 days elapsed between the catheterization procedures and the experiments On the day of the experiment a catheter was also inserted into a femoral artery The arterial catheter was kept patent by regular flushing with saline

After an overnight fast 30 ml trioctanoin (C8 0) was given by mouth Blood glucose FFA and IRI were determined simultaneously in portal and arterial blood before trioctanoin was given and 30 60 90 and 120 min thereafter

For comparison of mean values Student s *t* test was used This test was also applied to paired observations

RESULTS

The basal glucose concentration in arterial blood was 87±8 mg/100 ml and the corresponding value in portal blood was 85±7 mg/100 ml (mean±S E M) The difference between these mean values was not statistically significant In one patient (no 6) the fasting glucose concentration was slightly elevated both in arterial and in portal blood (116 and 117 mg/100 ml respectively) In the remaining patients the concentration was below 100 mg/100 ml (Table II) No significant change in the glucose concentration was found after ingestion of trioctanoin

Mean fasting FFA in arterial blood was 0 56±0 11 mEq/1 000 ml which is close to the mean (0 55±0 08 mEq/1 000 ml) found in a group of healthy subjects recently investigated in this laboratory (3 8) Although mean fasting FFA was 20% higher in the portal blood (mean ± S E M =0 67±0 10 mEq/1 000 ml) this difference was not statistically significant After administration of trioctanoin the portal FFA was significantly elevated at 30 and 60 min (Table II) From Fig 1 it will be seen that the mean portal FFA rose 76% during the first 30 min after fat ingestion It then stayed on a plateau until 90 min after which it fell

Mean basal IRI levels in arterial and portal blood were 6±2 and 11±2 µU/ml respectively ($p>0$ 05) A significant rise in the portal IRI was recorded 30 60 and 90 min after trioctanoin had been given (Table II) The mean portal IRI peaked at 60 min at 90% above the basal level (Fig 1) In the arterial blood there was no significant change in the IRI level after fat ingestion as shown in Table II

DISCUSSION

In the present investigation oral administration of MCT to five patients with pancreatitis and to one patient with carcinoma of the pancreas resulted in a small but significant increase in the portal IRI (Table II and Fig 1) Both the portal and the arterial glucose level remained practically unchanged after the fat ingestion (Fig 1) In this respect the findings are in accordance with previous observations indi

cating that MCT may influence insulin secretion without affecting the blood glucose concentration (5 7)

Sanbar and Martin (9) have demonstrated that octanoic acid stimulates the release of insulin from an in vitro rat pancreas preparation presumably by a direct effect on the β-cells In man little is known about the way in which octanoic acid may influence these cells It has been shown however that intraduodenal infusion of glucose and octanoic acid results in higher serum insulin levels than those obtained after intraduodenal glucose alone (6) Furthermore McCullough et al (7) have reported that MCT induce a significant increase in the peripheral IRI in patients with cirrhosis of the liver and portal systemic communications but not in cirrhotics without such communications Therefore these authors have suggested that small increments in insulin secretion induced by MCT might probably be masked by first circulation binding and removal by the liver The present data support this suggestion since the portal IRI increased significantly after ingestion of trioctanoin whereas no distinct change was obtained in the arterial blood (Table II Fig 1)

The significant rise in the portal FFA after trioctanoin ingestion was probably due to intestinal ...rption of octanoic acid followed by transport to ... liver via the portal vein Hepatic retention of ... might then have occurred since the arterial ...A did not change significantly after fat ingestion (Table II Fig 1) In this connection however it must be mentioned than an increased dilution of FFA in the systemic blood might also have contributed to the unchanged arterial level of FFA after fat ingestion

Although no significant rise in arterial FFA was obtained after MCT in the present investigation this does not exclude the possibility of changes in the arterial octanoate concentration causing an elevation of the portal IRI Furthermore it remains to be resolved whether ingestion of trioctanoin might have caused a release from the intestinal mucosa of hormones such as secretin pancreozymin or gut glucagon which are all known to affect insulin secretion

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The Influence of Diabetic Regulation on the Diurnal Variation in Blood and the Urinary Excretion of Ketone Bodies

Studies in Newly Diagnosed Diabetics

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ABSTRACT A typical diurnal variation of blood ketone bodies (acetoacetate+3 hydroxybutyrate) has been found in 23 newly diagnosed, untreated diabetics regardless of diabetic type It was characterized by high morning concentrations, followed by a fall, and later on a rise in concentration during the evening and night Satisfactory diabetic regulation judged from glucose measurements did not change this diabetic pattern, which differed fundamentally from the normal diurnal variation In the regulated state the morning concentration of ketone bodies in blood was still abnormally high in the juvenile and the non-obese maturity onset diabetics, while the concentration in the phenformin treated diabetics had increased on each sampling time The 24 hour urinary excretion of ketone bodies also remained abnormally high in the phenformin treated patients

In previous investigations (9 10) we have shown a characteristic diurnal variation in the concentration of ketone bodies in the blood of normal persons It is not known whether a similar variation in ketone body concentration is present throughout the day in newly diagnosed untreated diabetics or how diurnal variation in these patients is influenced by different forms of diabetic treatment The present investigation was undertaken to study these problems In addition observations were made on the relationship between ketone bodies and glucose in blood and urine

MATERIAL AND METHOD

The study was performed in 23 newly diagnosed diabetics and 17 control persons without diabetes The diabetic group consisted of 8 non-obese juvenile diabetics (aged 15–51 years mean 33) 8 non-obese maturity onset diabetics (aged 34–81 years mean 57) and 7 obese maturity onset diabetics (aged 45–74 years mean 63) In the control group 10 were non-obese (aged 25–63 years mean 40) and 7 obese (aged 49–80 years mean 61) Mean degree of overweight (4) in the obese maturity onset diabetics was 38% (range 18–68) in the obese controls 33% (range 23–55) The controls had normal plasma glucose throughout the day no family history of diabetes and had been admitted to hospital because of minor diseases of the locomotor system or neurotic disorders

Treatment

The juvenile diabetics and the non-obese maturity onset diabetics were given a diet containing about 2 000 calories (100 g protein 190 g carbohydrate and 85 g lipid) together with insulin or glibenclamide respectively Insulin 12–21 U was given in the morning All the juvenile diabetics received isophane insulin and two regular (crystalline) insulin also The dose of glibenclamide varied between 2 1/2 and 20 mg The obese maturity onset diabetics were treated with a 1 200 calories diet (105 g protein 105 g carbohydrate and 40 g lipid) together with phenformin 50–100 mg daily They had breakfast at 8 a m lunch at 12 noon and dinner at 5 30 p m At 2 p m and 8 p m they were given bread and coffee

Experimental procedure

The diabetics were studied during two 24-hour periods the first on the day after admission (on the 2 000 calories diet) before treatment with insulin or oral antidiabetic drugs the second during treatment after the best possible regulation had been obtained During each study period determination of acetoacetate (AA) 3 hydroxybutyrate (3 HB) and glucose in venous blood was performed 6 times The blood samples were taken at 8 a m before breakfast at the beginning of the study period at 11 a m 3 p m 9 p m 12 p m and 8 a m at the end of the study period The urinary excretion of AA 3 HB and glucose was determined during one or two 24-hour periods before

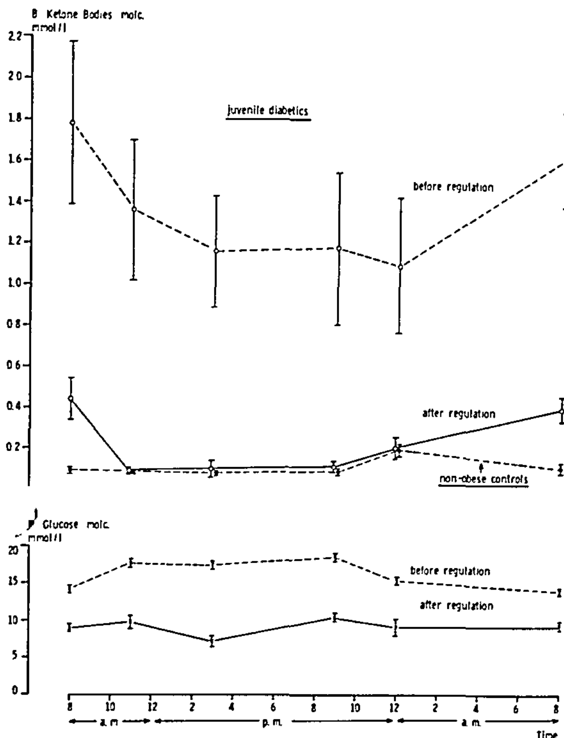


Fig. 1. Blood ketone bodies (acetoacetate+3 hydroxy butyrate) in non-obese controls (● ●) and juvenile diabetics before (O- -O) and after (O—O) diabetic regulation and plasma glucose in the diabetics before (× ×) and after (×—×) regulation (mean ± S.E.M.).

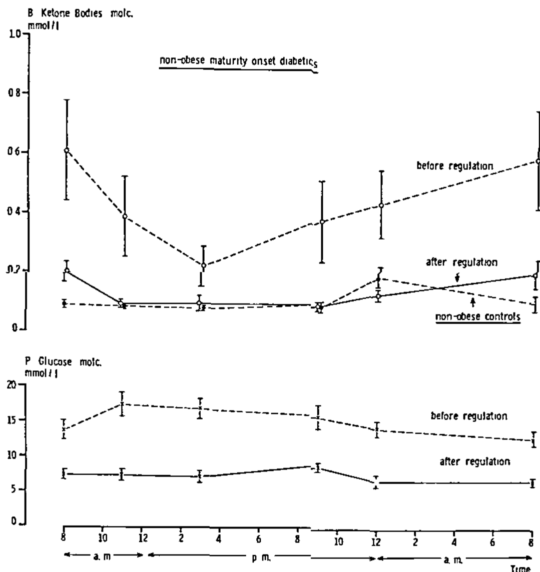


Fig 2 Blood ketone bodies (acetoacetate+3-hydroxy butyrate) in non-obese controls (● ●) and non-obese maturity onset diabetics before (O O) and after (O—O) diabetic regulation and plasma glucose in the diabetics before (× ×) and after (×—×) regulation (mean ±S E M)

as well as after regulation After voiding the urine was immediately placed in sealed containers at 4°C until analysis

Similar 24-hour studies were carried out in the controls who were given the 2 000 calories diet on the day of investigation In the obese controls the investigation was repeated after a 1 200 calories diet for 7 days

During the study periods the patients were not allowed to smoke and they were confined to the ward Activity was otherwise unrestricted They took an afternoon nap from 12 30 to 1 30 p m and retired for the evening at 9 p m *AA and 3 HB in blood and urine were measured* using an enzymatic micromethod (8) The ketone body concentration was calculated by summation of the concentrations of AA and 3 HB The concentration of glucose in plasma and urine was determined by an o-toluidine method (1) In the statistical analysis the Wilcoxon test for paired differences the Wilcoxon test for two samples and Spearman s rank correlation were used

RESULTS

Blood ketone bodies and plasma glucose

In Figs 1 2 and 3 the mean ketone body concentration in blood (±S E M) and the mean plasma glucose concentration (±S E M) are given for each

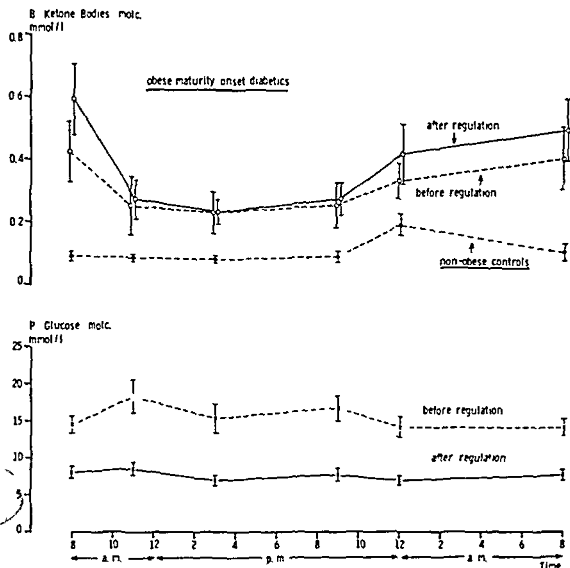


Fig 3 Blood ketone bodies (acetoacetate+3 hydroxy butyrate) in non-obese controls (● ●) and obese maturity onset diabetics before (O- -O) and after (O——O) diabetic regulation and plasma glucose in the diabetics before (× ×) and after (×——×) regulation (mean ±S E M)

sampling time in the diabetics before and after regulation together with the mean ketone body concentration in blood (±S E M) in the non-obese controls

In 9 of the non-obese controls the diurnal variation in blood ketone bodies was characterized by a significant rise in ketone body concentration from 9 p m to midnight followed by a significant decrease in concentration from midnight to 8 a m

In each group of diabetics on the other hand the highest concentrations were found in the morning before as well as after regulation the mean morning concentration at the beginning and the end of the study period being significantly higher ($p<0.05$) than the mean concentration during the rest of the day

Before treatment the mean ketone body concentration in the juvenile and in the obese maturity onset diabetics was significantly higher than in the non-obese controls for each sampling time ($p<0.05$) In the non-obese maturity onset diabetics mean ketone body concentration was significantly higher at 8 a m 11 a m and 9 p m ($p<0.05$)

After regulation a significant decrease in the concentration of blood ketone bodies was seen in the juvenile diabetics and in the non-obese maturity onset diabetics ($p<0.05$) Compared to the non obese controls the concentration in both these

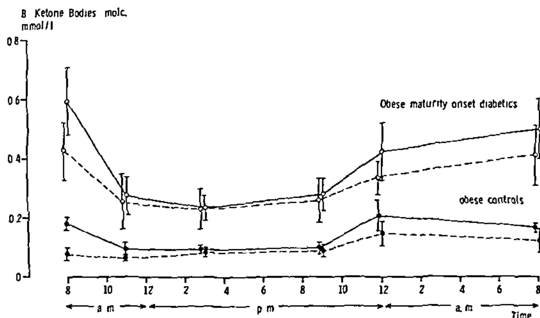


Fig 4 Blood ketone bodies (acetoacetate+3 hydroxy butyrate) in obese maturity onset diabetics before (O O) and after (O——O) diabetic regulation and in obese controls before (● ●) and after (●——●) 1200 calories diet (mean±S E M)

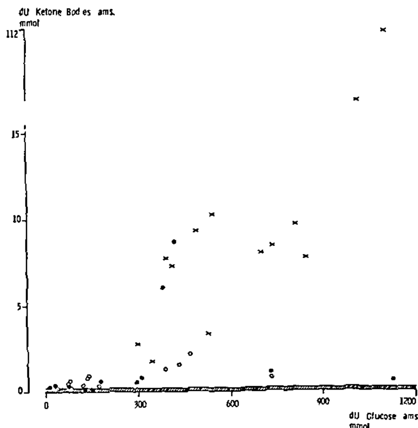


Fig 5 Correlation between 24 hour urinary excretion of ketone bodies (aceto-acetate+3-hydroxybutyrate) and glucose in juvenile diabetics (×) non-obese (●) and obese (O) maturity onset diabetics before diabetic regulation The hatched area indicates the mean 24-hour urinary excretion of ketone bodies ±2 S D in the non-obese controls

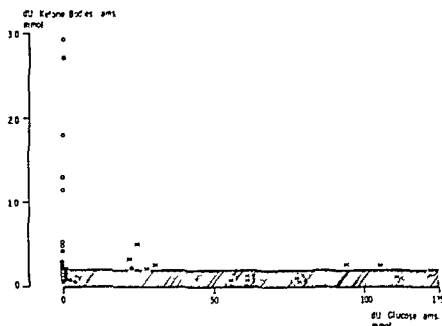


Fig 6 Correlation between 24 hour urinary excretion of ketone bodies (acetoacetate + 3-hydroxybutyrate) and glucose in juvenile diabetics (×) non-obese (●) and obese (O) maturity onset diabetics after diabetic regulation The hatched area indicates the mean 24 hour urinary excretion of ketone bodies ±2 S D in the non-obese controls

groups of diabetics was significantly higher only in the morning ($p<0.01$) Contrariwise in the obese maturity onset diabetics a slight but non significant increase in mean ketone body concentration was seen after regulation and the mean concentration for each sampling time was still significantly higher than in the non obese controls ($p \leq 0.02$) Compared to the obese controls given the 1 200 calories diet for 7 days the mean concentration of blood ketone bodies in the regulated obese diabetics was 2-3 times higher the difference being significant ($p \leq 0.02$) for each sampling time except midnight (Fig 4) The restriction in caloric intake in the obese controls caused a slight increase in mean ketone body concentration (Fig 4) significant only in the morning ($p=0.05$)

There was no significant correlation between the concentrations of ketone bodies in blood and glucose in plasma in any of the three groups of diabetics neither before nor after regulation

The urinary excretion of ketone bodies and glucose

The relationship between the 24 hour excretions of ketone bodies and glucose in urine in the diabetics before treatment is given in Fig 5 together with the normal 24 hour urinary excretion of ketone bodies i e the excretion in the non-obese controls which was 98±112 µmol (mean±2 S D) In each group of diabetics the excretion of ketone bodies was higher than the normal excretion The correlation between the excretion of ketone bodies and glucose in urine was significant in the juvenile diabetics ($p<0.01$ $R=0.769$) in the non-obese maturity onset diabetics ($p<0.02$ $R=0.720$) and in the obese maturity onset diabetics ($p<0.02$ $R=0.720$)

Fig 6 shows the relationship between the 24 hour urinary excretions of ketone bodies and glucose after regulation for the same persons In the juvenile diabetics the excretion of glucose was less than 120 mmol the excretion of ketone bodies being nearly normal There was no significant correlation In the maturity onset diabetics the urine was practically glucose free While the urinary ketone body excretion in the non-obese maturity onset diabetics was nearly normal the excretion of ketone bodies in the majority of the obese diabetics exceeded the normal values considerably In one patient excretion was 14 times the highest normal limit In none of the patients could this augmented urinary ketone body excretion be revealed using Acetest®

DISCUSSION

In normal persons the most typical features of the diurnal variation in blood ketone bodies are low morning concentrations and a ketone body peak around midnight (9 10) The present study has revealed a fundamentally different pattern in blood ketone bodies in newly diagnosed untreated diabetics regardless of diabetic type This diabetic pattern is characterized by the highest concentrations

in the morning followed by a fall in concentration and later on a rise in concentration during the evening and night Diabetic regulation does not change this typical pattern A similar pattern in breath acetone concentrations has earlier been reported in insulin-dependent diabetics (7)

In the obese diabetics a rise in the blood concentration of ketone bodies and in the 24 hour urinary excretion of ketone bodies occurred during phenformin treatment in spite of satisfactory diabetic regulation This resistant ketosis could not be due to the low calorie diet they were given as the ketone body concentration in blood was significantly higher than in the obese non-diabetic persons given the same diet Thus phenformin did not have a lowering effect on the concentration of blood ketone bodies and the increased ketosis during treatment might have been due to phenformin This effect of phenformin has been demonstrated in normal starved subjects (3) and in phenformin treated maturity onset diabetics Kattermann et al (2) found a significant increase in blood ketone bodies in morning fasting specimens

Compared to the non-obese controls blood ketone bodies in the juvenile diabetics and the non-obese maturity onset diabetics after diabetic regulation were significantly higher only in the morning This finding indicates that insulin as well as glibenclamide should be given twice instead of once a day to decrease morning hyperketonaemia In labile juvenile diabetics Åkerblom and Hiekkala (11) in accordance with this observed a significant decrease in morning hyperketonaemia by shifting from 1 to 2 injection insulin therapy

This study has shown that patients during satisfactory diabetic regulation as judged from glucose measurements still had an abnormal ketone body regulation This was the case in both juvenile diabetics and maturity onset diabetics It must be emphasized that the sensitivity of Acetest® to detect ketonuria was not great enough to reveal the demonstrated abnormalities in ketone body metabolism Consequently to achieve better diabetic regulation determination of the concentration of ketone bodies in blood and the 24 hour urinary excretion of ketone bodies should be included in the control of insulin treated diabetics (5 6) as well as in diabetics treated with oral antidiabetic drugs

ACKNOWLEDGEMENTS

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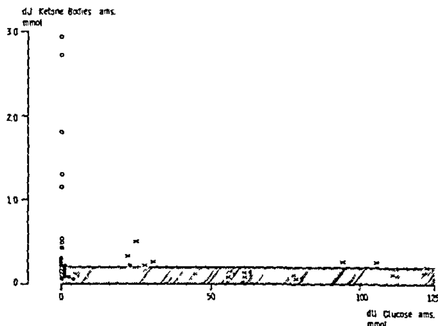


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The Role of Lymphography in the Investigation of Patients with Fever of Unknown Origin

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Abstract Lymphography has been performed in 21 patients with fever of unknown origin In 8 of the patients lymphography was positive demonstrating retroperitoneal lymphoma. The value of lymphography before planning a laparotomy is stressed

One of the initial symptoms of a malignant disease is often fever either prolonged or periodic of the Pel Ebstein type This is particularly true in malignant lymphomas The suspicion of malignant lymphoma in patients with prolonged fever is strong and it is mentioned as one of the indications for lymphography (2 4 6 10 11) In order to determine the role of lymphography as a diagnostic tool in the investigation of patients with fever of unknown origin a survey has been made of a material in which extensive clinical investigations had failed to give the diagnosis before lymphography was performed

MATERIAL AND METHODS

The material consists of 21 patients 9 women (aged 31–70 mean 54) and 12 men (aged 19–64 mean 46) Two patients had high continuous fever two had a prolonged subfebrile temperature and 16 had attacks of high fever followed by non-febrile periods of varying length One patient had high fever almost every night 1/2 hour after going to bed The febrile state had lasted for 1–15 months before lymphography was performed

The clinical examination revealed no palpable tumors or pathologic lymph nodes The roentgenologic investigations consisted in most cases of a total examination of the gastrointestinal tract lungs urography and in approximately half of the patients of the nasal sinuses teeth skeleton and abdomen Angiography of either the renal superior mesenteric or coeliac arteries was performed in 12 patients Sternal puncture was made in 17 patients aspiration biopsy of the liver in 14 and of the spleen in 9 Biopsy of muscle tissue was performed in 6 patients of skin in 2 of temporal artery in 4 of lymph nodes in 9 and of rectal mucosa in 3 patients Various bacteriologic cultures and serologic tests had given no explanation for the fever

The lymphographies were performed according to the modification of the Kinmonth technique described by Wallace et al (12)

RESULTS

In 8 patients lymphography revealed a malignant retroperitoneal lymphoma Histology was determined by laparotomy in 3 patients by aspiration biopsy of the spleen in 2 and by lymph node biopsy in 2 no histologic verification could be obtained in one patient The lymph nodes that were biopsied did not become apparent and palpable until after lymphography Histology revealed Hodgkin s disease in all the lymphomas but in one the diagnosis was later changed at autopsy to myeloid leucemia (the initial histologic diagnosis was determined by aspiration biopsy of the spleen) In the remaining 13 patients with normal lymphographies the final diagnoses were fever of unknown etiology 8 status post hepatitis 1 arteritis rheumatica 1 virosis cytomegalica 1 gastrointestinal allergy 1 metastases to the spine 1 (unknown primary tumor not found at autopsy)

DISCUSSION

In patients with fever of unknown etiology extensive clinical investigations are undertaken in order to find or rule out malignant disease collagenosis or an inflammatory process The use of lymphography in these patients is not very

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common according to the literature However Chiappa et al (1) describe 3 patients with suspected malignant lymphoma which could be demonstrated only with lymphography Two of these patients had a long standing fever as the dominant symptom Domart et al (3) report 13 patients with fever in whom lymphography was performed The patients were divided into three groups 1) already known malignant lymphoma where the extension of the disease was to be determined (1 patient) 2) fever and adenopathy where the histology was not clear (4 patients) or where biopsy had not yet been performed for instance in tumors of the mediastinum (3 patients) 3) unexplained fever without local symptoms or palpable nodes (5 patients) In the third group lymphography revealed one patient with Hodgkin s disease

Fever is a common symptom in Hodgkin s disease (9) and lymphography should therefore be considered quite early in the investigation It must be born in mind however that lymphography has disadvantages lung complications are reported and even deaths but when the amount of contrast medium is kept to a minimum the rate of complications is very low Another disadvantage is that lymphography does not reveal involvement of the lymph nodes in the hilum of the liver and spleen or the mesenteric lymph nodes

mphography has a two fold purpose in these The first is diagnostic to reveal or to rule out lymphoma In the event of positive findings i e malignant lymphoma a laparotomy and lymph node biopsy are necessary for histological verification In these cases lymphography has its second purpose to guide the surgeon as the malignant lymph nodes may be quite discrete and may escape even a trained eye A correct staging laparotomy in Hodgkin s disease also includes wedge biopsy of the liver and splenectomy (7) Lymphography should therefore be performed before planning a laparotomy (5 8) to indicate a proper technique for the operation

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Hypothyroidism with Subacute Pseudomyotonia – an Early Form of Hoffmann's Syndrome?

Report of a Case

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ABSTRACT A 25-year-old man was admitted to the hospital because of painful muscle cramps and action myospasms of subacute onset and of 6 weeks' duration No myotonia could be demonstrated objectively and his deep tendon reflexes showed no prolongation of the relaxation phase Serum creatinine was raised but creatinine clearance was normal Serum levels of aldolase CPK ASAT and ALAT were increased but ordinary light microscopy revealed no histological signs of muscle disease in a quadriceps biopsy ECG showed a prolonged PQ interval and flat T waves in the left precordial leads Laboratory tests of thyroid function revealed intensive hypothyroidism and high titers of circulating thyroid antibodies were demonstrated During $2^1/_2$ months of thyroid therapy the muscle symptoms gradually disappeared completely and the patient could return to work By that time the serum enzymes and the ECG had normalized Despite the lack of objective signs of myotonia, we consider that the very dominant subjective muscle symptoms, severe enough to prevent the patient from performing his ordinary manual work and completely reversible on thyroid therapy, justify the designation of hypothyroid myopathy The question is raised whether the case represents an early form of Hoffmann s syndrome

Hypothyroidism is often accompanied by mild muscle symptoms such as increased volume stiffness slowness of contraction and slowness of tendon reflexes In rare cases marked muscle symptoms may be present The clinical picture may then simulate congenital myotonia or myotonic dystrophy Cretinism manifesting such a muscle picture is called Debre Semelaigne syndrome and similar muscle symptoms accompanying myxedema are designated Hoffmann s syndrome (6)

The present paper describes the clinical and laboratory findings in a young man with severe hypothyroidism presenting clinically with subacute pseudomyotonia

CASE REPORT

A 25 year-old man was admitted to the hospital on Sept 2 1974 because of painful muscle cramps and action myospasms of approximately 6 weeks duration Up to a few weeks before the onset of these muscle symptoms he had been playing football without difficulty The cramps and myospasms had occurred with increasing frequency and severity in the arms the legs the chest and the abdomen predominantly with movement Finally the patient had to stop doing his ordinary manual work and consulted a general practitioner who referred him to our hospital with the diagnosis of suspected dystrophia myotonica

On physical examination his voice was deep his face somewhat expressionless and his skin dry He had no goiter and the thyroid was not tender The BP was 130/95 The deep tendon reflexes were normal No prolongation of the relaxation phase could be demonstrated Objectively no myotonia could be demonstrated but the patient complained of a feeling of stiffness when stretching his fingers after firm closure of the fists

Laboratory investigations yielded following results ESR 4 mm/h Hb 16 0 g/100 ml hematocrit 44% leucocytes 8 100/mm^3 Potassium 3 65 sodium 139.5 and calcium 4 6 mEq/l Creatinine range 1 6–1 9 mg/100 ml There was no proteinuria and the sediment was normal Creatinine clearance was 96 4 ml/min Bilirubin was 0 62 mg/100 ml alkaline phosphatase 31 mU/ml (20–48) ASAT range 59–70 (below 40) ALAT 68–48 (below 40) and LD 430–504 U/l (below 475) Cholesterol was 350 mg/100 ml triglycerides 2 15 mmol/l and lipoprotein electrophoresis showed increased β-lipoprotein Plasma protein analysis was normal

Creatinine phosphokinase (CPK) was 564 IU/l (0–100)

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and aldolase 4 2 mU/ml (0.5–3 1) T_4 iodine was 0 3 μg/100 ml (2 4–6 0) T_3-sephadex test 70% (80–120) and TSH more than 50 mU/l (1 5–7 2) Antibodies were demonstrated in a titer of 1/400 against thyroglobulin and in a titer of more than 1/100 against cytoplasmic thyroid antigen ANF test was negative

Light microscopy of a quadriceps biopsy revealed no histological signs of muscle disease Liver biopsy showed minimal fatty change ECG showed a PQ interval of 0 26 sec and flat T waves in the left precordial leads at a heart rate of 65/min X ray of the chest was normal The size of the cardiac silhouette was 910 ml corresponding to 410 ml/m² BSA

The diagnosis of suspected Hoffmann s syndrome was made and treatment with levothyroxine was started The muscle symptoms gradually disappeared After two months therapy the daily dose of levothyroxine had been raised to 0 3 mg and the T_4 I was 5 2 μg/100 ml One month later the following values of serum enzymes were obtained CPK 70 ASAT 23 ALAT 22 U/l A new ECG showed that the PQ interval had been reduced to 0 18 sec and that the T wave changes in the left precordial leads had disappeared at a heart rate of 55/min By that time the pseudomyotonic symptoms had completely vanished and the patient could return to work Since then he has been able to perform his ordinary manual work regularly for an observation period of two months without any recurrence of muscle symptoms The levothyroxine dose has been retained at 0 3 mg daily

DISCUSSION

patient is described in whom intensive hypo… presented clinically with pseudomyo… of subacute onset and of such severity that had to stop doing his ordinary manual work Subjectively the patient experienced painful muscle cramps and action myospasms Yet no myotonia could be demonstrated objectively and the deep tendon reflexes showed no prolongation of the relaxation phase

The very low level of T_4 I and the very high level of TSH indicate a severe degree of primary hypothyroidism The high levels of circulating thyroid antibodies point to an autoimmune thyroiditis as the cause of the myxedema The high level of CPK and the increased levels of aldolase ASAT and ALAT indicate muscle disorder but ordinary light microscopy of the muscle biopsy revealed no histological signs of muscle disease Increased serum concentrations of CPK ASAT and LD are frequently seen in hypothyroidism (4) and thus do not as such differentiate our case from ordinary cases of hypothyroidism Theoretically the liver involvement as shown by the minimal fatty change demonstrated in the liver biopsy could also have contributed to the raised levels of ASAT ALAT and LD in our case All other liver function tests were normal however

The important differentiating aspect of our case is the manner in which the hypothyroidism presented clinically The muscle symptoms developed over a short period and became severe enough to prevent the patient from performing his ordinary manual work The painful muscle cramps and action myospasms were the only subjective complaints, leading the diagnostic thinking of all doctors involved in the direction of a subacute muscle disease Although many patients with hypothyroidism complain of stiff aching muscles (4) we have never encountered any previous patient with such completely dominant muscle symptoms The absence of histological signs of other muscle disease and the complete clinical and laboratory restitution on thyroid therapy do strongly support the diagnosis of hypothyroid myopathy

Reports in the literature of cases of myxedema myopathy are rare Salick et al (5) and Ahuja (1) reported one case each Both patients had experienced muscle symptoms for several years prior to diagnosis and treatment In both muscle biopsies revealed increased numbers of sarcolemmal nuclei In the case of Salick et al occasional necrotic fibers were also found and in that of Ahuja abundant amorphous substance was reported In contrast to these two cases our patient had experienced muscle symptoms for some 6 weeks only prior to the muscle biopsy Whether this difference in duration of hypothyroidism can account for the fact that histological signs of muscle disease were lacking in our case remains to be established

Fessel (3) reported three hypothyroid patients who presented with muscle symptoms In two of them however no improvement of muscle function was seen on thyroid treatment and in the third improvement was seen only after adrenal steroids had been added to the regimen It seems questionable therefore whether hypothyroidism was really the cause of the muscle symptoms in these three cases

In 1969 Bondy (2) reported that he had only seen one patient who probably had myxedema myopathy He concluded that true hypothyroid myopathy must be a very rare complication of myxedema In our opinion the case presented by us represents such a very rare manifestation of hypothyroidism We would like to suggest that our pa

tient suffered from an early form of Hoffmann s syndrome The pathogenetic mechanisms of muscle dysfunction in such cases remains to be establ shed

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Fig 1 Pretreatment jejunal biopsy The villi are coarse and the lamina propria densely infiltrated with PAS positive macrophages



Fig 2 Jejunal biopsy following one year s treatment Normal appearing villi and few faintly staining macro phages in deeper part of lamina propria (arrow)

400 mg sulphamethoxazole and 80 mg trimethoprim After a few days treatment the general wellbeing improved considerably appetite reappeared and diarrhoea sub sided In two weeks he gained 4 kg in weight No folate or vitamin B_{12} supply was given

After two months medication the patient was in an llent state body weight 56 kg The bone marrow cobalamines and serum folates were normal In biopsies a considerable improvement was found ı less extensive infiltration of PAS-positive cells than before Subjectively he felt sound and was restored to work

Further regression in the histological changes had oc curred after six months treatment Haematological values were normal there was no steatorrhoea and absorption tests gave normal values (Table I) The drug was well tolerated and after one year s treatment the jejunal mu cosa had become almost normal The villi were slender and a very small number of faintly PAS-positive macro phages were observed in the deeper part of the lami na propria No malabsorption was present and the haematological status was normal (Fig 2 and Table I) The treatment was discontinued and at out-patient control three months later he is still in perfectly good health

Table I *Data of the patient before and after sulphamethoxazole trimethoprim treatment*

	Normal	Before treatment	After treatment for 6 mo	12 mo
Hb (g/100 ml)	>12 0	10 5	14 5	15 3
MCV (fl)	82–105	91	85	95
Leucocytes (10^3/μl)	<10 0	11 2	9 8	8 3
S-cobalamines (pmol/l)	>280	245	415	535
S-folates (ng/ml)	>2 0	1 8	3 8	3 0
Fecal lipid excretion (g/24 h)	<4 0	5 4	3 2	3 4
Schilling I (%)	>10 0	3 0	16 8	11 0
Schilling II (%)	>7.5	4 0	18 0	10 0
TRIFA test (%)	>30 0	26 0	38 0	55 0
Bone marrow		Megalo blastic	Normo blastic	Normo blastic
Weight (kg)		42 0	58 0	59 0
Stools (no /24 h)		6–9	1	1–2

DISCUSSION

All of the patient s symptoms could be ascribed to Whipple s disease the diagnosis of which was confirmed by jejunal biopsy

Generally Whipple s disease is considered to be a bacterial disease but as the infective agent is still unknown the choice of therapeutic substances is solely based on empiric grounds In the present case the clinical effect of sulphamethoxazole–trimethoprim was rapid and dramatic but a considerably longer period of treatment was needed before the villi had regained their normal structure This is in agreement with the observation that antibiotic treatment of Whipple s disease has to be continued for at least 10–12 months (2) Probably *repeated jejunal biopsy is the only way to record the* efficiency of the therapy instituted

The megaloblastic transformation in the bone marrow must undoubtedly be ascribed to deficiency of folate and vitamin B_{12} due to a reduced intestinal absorption of folic acid and vitamin B_{12} The cause of this malabsorption must be related to Whipple s disease itself as the intestinal absorption of folate and vitamin B_{12} was normalized in conjunction with regression of the intestinal lesions No side-effects of the sulphamethoxazole–trimethoprim therapy were observed

The bactericidal effect of this treatment is due to a blockade of the synthesis of folates in microorganisms Although folate status in man generally is unaffected (7) *precautions should be taken in* the folate depleted organism (4) unless remission occurs as rapidly as in the present case

We cannot assess the optimal therapy for Whipple s disease from a single case but we think that a sulphonamide combined with trimethoprim should be considered as the drug of first choice in the treatment of this rare condition

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REVIEW ARTICLE

Extracapillary (Crescenteric) Glomerulonephritis

It is now well recognized that uremia following primary renal disease in a majority of cases is due to one of several types of glomerulonephritis (GN) Although impressive progress (19) has been made mainly from studies of experimental GN in animals and by immunofluorescence and ultrastructural research in man (8) much remains in the understanding of pathogenic mechanisms leading to progressive glomerular disease

In the heterogeneous group of glomerulonephritides one type is defined morphologically by the presence of crescent like proliferation between Bowman s capsule and the glomerular tuft Volhard and Fahr (30) recognized this lesion in a minority of patients with acute nephritis and for this condition they coined the term subacute glomerulonephritis which they considered as invariably fatal leading to uremia and death within months

In more recent times it has been realized that glomerular crescents may be present in several distinct clinical entities including A) Primary GN 1) post streptococcal 2) non streptococcal 3) Goodpasture s syndrome B) GN as part of systemic disorders 1) systemic lupus erythematosus 2) polyarteritis nodosa 3) Schonlein–Henoch syndrome 4) Wegener s granulomatosis

An old term—extracapillary glomerulonephritis (EGN)—originally introduced by Fahr (10) has been readopted as a logical designation for this renal disease in which the outstanding light microscopical lesion is constituted by a cellular proliferation located to the space between the capillary tuft and Bowman s capsule Because this cellular mass is confined to the urinary space outside the vascular pole sections through a corpuscle will often appear as the shape of a cellular crescent The formation of crescents is probably a valid expression of the severity and extension of the inflammatory proliferation which in milder cases may lead to only smaller epithelial adhesions between the tuft and a limited segment of the capsule but also at the other end of the scale to massive proliferation completely surrounding the capillary tuft like a full circle broad band like structure

Additional lesions include mesangial cellular proliferation with narrowing of capillaries usually infiltration with polymorphonuclear leucocytes and in recent cases nearly always the presence of fibrin These changes are to some extent dependent on the stage of development of the disease and some of them significantly related to the duration of the disease process (23) The relative proportion of sclerotic glomeruli and the amount of collagen fibrils in crescents and interstitial fibrosis are more severe in later stages while the cellularity of crescents the number of neutrophils and the amount of fibrin diminish with the duration Other features present in all types of EGN are multinuclear giant cells and large defects of basement membranes of Bowman s capsule as well as of the capillary tuft

The natural history of development of the crescenteric structure is well known in cases of progressive failure and fatal outcome In such patients the cellular proliferation is gradually replaced by fibrotic tissue leading to partial or complete sclerosis of the corpuscle The fate of crescents in non fatal cases due to spontaneous recovery or remission induced by therapy has been studied in only a limited number of patients It does appear however that crescents may disappear entirely or nearly so leaving only small adhesion of fibrous tissue (7 18)

When different clinical groups of EGN are compared (23) no differences in glomerular pathology by light microscopy can be discerned indicating that differentiation between these groups cannot be done by studying biopsy material only Possible

exceptions are Wegener s granulomatosis and poly arteritis nodosa although necrotizing arteritis similar to that of polyarteritis has been described in post streptococcal EGN (7)

While in EGN nearly all or a great majority of glomerular cross sections of a specimen contain cellular masses in the urinary space sporadic or solitary crescents may occur in a variety of renal disorders of clearly non-glomerular origin such as renal infarcts pyelonephritis and malignant nephro sclerosis For this reason it has been debated how large a proportion of glomeruli should contain crescents before a diagnosis of EGN is justified Figures from 33% and upwards have been suggested as a criterion or prerequisite for this disease Although this decision may be arbitrarily founded we believe that crescents in more than three out of ten glomeruli in a biopsy specimen strongly suggest EGN unless additional morphological and clinical findings indicate otherwise

Recent works on the glomerular ultrastructure *have revealed lesions not previously known* (4 6 20 22) Studies of the composition of cellular elements of crescents have suggested multiple origins and not only as previously held a proliferation of capsular and visceral epithelium Features characteristic for monocytes and macrophages and gradual transitions to cells reminiscent of fibrocytes [illegible] *seen mostly in material from patients with* [illegible] duration of the disease Such foreign cells could possibly penetrate from without through the defects of Bowman s capsule but one cannot exclude the possibility that they represent specially differentiated glomerular epithelial cells One type of ultrastructural lesion is very impressive in many glomeruli there is extensive destruction of the basement membrane both in the capillaries and in that of Bowman s capsule (23) These defects are so common that they possibly occur in all glomeruli with crescents although not necessarily represented in all sections It is a peculiar fact that the basement membrane shows a nearly normal structure right up to the edge of the defect without clear changes of the fine structure When defects of the glomerular capillary basement membrane are present the adjoining capillary lumen is often thrombosed

Immunopathologic studies of EGN with fluorescing antibodies have not hitherto yielded conclusive evidence concerning pathogenic mechanisms Linear and non linear patterns of immune deposits as well as cases with no deposits at all have been reported by several authors (2 3, 9 11 14 15 16 17 21 24 25 26 27) EGN in Goodpasture s syndrome was earlier believed to be associated with linear patterns indicating deposits of antiglomerular basement membrane antibodies More recently however non linear patterns suggestive of immune complex disease were reported (3 16 17) The absence of immune deposits in some cases of Goodpasture s syndrome as well as in other clinical entities of EGN is difficult to reconcile with an immunologically mediated lesion unless the presence of deposits in such cases were short lived In this context it may be mentioned that the basement *membrane defects in our studies (4) often considered* as resulting from immunologic injury were not usually associated with adjacent ultrastructural deposits In our view this severe destruction of the basement membrane is the most important lesion distinguishing EGN from other types of proliferative GN

Although direct evidence for an immuno pathogenic process may be absent in occasional cases such mechanisms are responsible in a majority of cases as the initiating event leading to cascade activation of the complement system and intravascular coagulation with fibrin formation Fibrin is nearly always present in the capillaries and *in the urinary space in human EGN* and has been demonstrated early in the production of experimental GN (28 29) It has been suggested from these studies that fibrin deposits may be a major factor responsible for crescent formation

Clinically EGN in a majority of cases runs a rapidly progressive course leading to extinction of *renal function within a short period* ranging from days to a few months The occasional usage of the term rapidly progressive GN as synonymous with EGN is however unfortunate partly because rapidly progressive GN may be associated with glomerular lesions without crescents partly because EGN may take a prolonged course particularly if part of systemic disease and finally because arrest may occur spontaneously or following therapy in cases where less than 50% crescents are present

In several series of EGN (1 12 13) a varying proportion of cases was associated with evidence of preceding streptococcal infection The frequencies stated are probably minimum figures as such evidence was not sought for in all cases also such

evidence may have disappeared at the time of admission It remains conceivable however that post streptococcal as well as non streptococcal categories of EGN occur The distinction between these two groups would seem important if it is correct that the prognosis as has been suggested (7 13) is better for post streptococcal EGN than in cases of idiopathic (non streptococcal) EGN with a similar severity of lesions In non streptococcal EGN the course and prognosis are related to the proportion of glomeruli affected by crescent formation and if more than 50–60% crescenteric glomeruli occur in a biopsy containing not less than 10 glomeruli it may be safely assumed that cessation of renal function will prevail

The present therapeutic possibilities include anti coagulants aimed at preventing intracapillary thrombosis and platelet aggregation usually combined with steroids and immunosuppressive agents Due to the high risk involved in using these drugs it would seem inadvisable to undertake such treatment without prior evaluation concerning possible post streptococcal etiology and particularly renal biopsy *with determination of the proportion and* extension of glomerular crescents Impressive results have been reported recently by quadruple chemotherapy (5) and combined steroid–azathioprine treatment (12) Controlled clinical trials have not appeared and would be difficult to accomplish due to the rarity and heterogeneity of the condition The rapidity of its course and the general outcome contribute to making such a procedure unnecessary The heterogeneity of etiologic and pathogenic factors however does require a precise clinical classification and follow up and particularly strict pathological criteria By such means it should be possible to obtain useful information concerning the efficacy of drug therapy

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Heme Catabolism during Short-term Treatment with Phenobarbital, Diazepam and Oxazepam

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ABSTRACT Carbon monoxide production (VCO) total body heme (TBH) and serum bilirubin (SB) have been determined in healthy young men before and after 100 mg phenobarbital (10 subjects), 15 mg diazepam (7 subjects) and 75 mg oxazepam (7 subjects) respectively, daily for seven days None of the drugs had any significant effect on VCO SB and TBH were also unaffected Baseline VCO (mean ±1 S E M) was 12 6±0 6 μmol/mmol TBH and day The postdrug VCO was 15 1±1 8, 14 1±1 4 and 14 7±1 5 after phenobarbital, diazepam and oxazepam respectively The corresponding values for SB were 5 4±0 8, 6 0±1.5 and 6 2±1 0 μg/ml compared to a baseline value of 6 6±0.8 μg/ml However, when the pooled postdrug data were compared with the pooled baseline values, mean VCO showed a probably significant increase (from 12 6±0 6 to 14 7±1 0 μmol/mmol TBH and day ($p<0\ 05$) It is concluded that although phenobarbital is known to increase the hepatic heme turnover, this effect is not measurable in terms of total heme turnover, no more than 20% of which comes from the liver

In 1970 Coburn (5) reported that the administration of 100 mg phenobarbital daily for seven days to five healthy adults increased the endogenous production of carbon monoxide (VCO) by 70% Simultaneously total body heme (TBH) measured with the CO dilution technique increased 10% Similar effects were seen after the administration of diphenylhydantoin in therapeutic doses Since the concentration of hemoglobin and the reticulocyte count did not change and since both drugs are known to be effective enzyme inducers (10) Coburn concluded that the changes represented catabolism of a markedly increased pool of heme-containing enzymes in the liver

The purpose of the present study was to elucidate the effect of phenobarbital on heme catabolism with the aid of a larger material and more frequent heme catabolism studies in some of the volunteers Furthermore we were interested to compare the effect of phenobarbital with that of two common bensodiazepines diazepam and oxazepam especially as Albanus et al (1) report that in the dog diazepam likewise has a marked enzyme inducing activity significantly different from that of oxazepam

MATERIAL

The subjects studied were 10 healthy male medical students aged 22–27 years (median 24) They were all known personally to at least one of the investigators They ate a normal diet and denied the use of any medication and abuse of alcohol Five of the subjects smoked 3–15 cigarettes daily but completely abstained from smoking for at least 36 hours before each study

In the first series all subjects were given 100 mg phenobarbital by mouth in the evening for seven days In the second and third series respectively seven of the subjects were given diazepam 5 mg three times daily and oxazepam 25 mg three times daily for seven days The weeks on the drugs were separated by at least two weeks free from drugs For details see Table I

Determination of VCO was always undertaken in the morning usually between 7 15 and 10 00 a m The subjects were then in the postabsorptive state and had not taken any food since 9 00 p m the day before They were told to get to the laboratory without exerting themselves and avoiding CO contamination from vehicles

METHODS

VCO was measured with a closed rebreathing system according to Coburn et al (6 7) After an equilibration period of 20 min blood samples were drawn every 15 min through an indwelling venous catheter EDTA was used as anticoagulant After 120 min (9 samples) 20 0 ml 99 9%

Table I *Subjects and plan of study*
D=diazepam O=oxazepam P=phenobarbital

Subject no	Age (y)	Weight (kg)	Drug series I	II	III	IV[a]
1	24	70	D	P	O	P
2	23	73	D	P	O	
3	22	65	P	O	D	
4	27	75	P	O	D	
5	24	70	P	O	D	P
6	25	80	P	O	D	
7	24	72	P	O	D	
8	22	80				P
9	23	57	P			
10	24	84	P			

[a] VCO TBH and SB were determined on days 0 2 4 and 7

pure CO (AB Alfax Malmö Sweden) were injected into the system a final sample being drawn after another 25 min The CO content of the blood samples was determined by gas chromatography as described by Collison et al (8) and Rodkey and Collison (22) modified as described by Lundh et al (17) 99 9% pure CO injected as described by Celegin et al (4) was used for calibration The Hb concentration was determined according to van Kampen and Zijlstra (13) The S D for a single analysis of COHb, calculated from duplicate analyses on 100 blood samples with a COHb varying between 0 46 and 4 61% was found to correspond to a coefficient of variation of % Duplicate analyses were always performed VCO l/h STPD) was calculated by the formula

$\dot{C}O$ (ml/h)=ΔCOHb×dilution factor

In this formula ΔCOHb is the hourly increase in the percentage saturation of Hb with carbon monoxide (COHb) determined from the COHb of the first nine blood samples using the least squares criterion to compute a best fit for the relation between time and COHb The dilution factor was determined from measurements of the increase in COHb ($\Delta COHb_{inj}$) after the injection of a known amount (20 0 ml) of CO (CO_{inj}) into the rebreathing system CO binding capacity (CO B C) was calculated from the dilution factor by the formula

$$CO\ B\ C\ (ml)=\frac{CO_{inj}\times 100}{\Delta COHb_{in}}$$

*Total body hemoglobin (TBHb g) was calculated by di*viding CO B C by 1 39 and TBH (mmol) by dividing TBHb by 16 1

The results of the VCO studies will be given in relative values µmol CO/mmol TBH and day This corresponds to the fraction of total heme mass destroyed per day used by Coltman and Dudley (9) and Lynch and Moede (18)

Immediately before the injection of CO into the rebreathing system an extra blood sample was drawn for the determination of serum bilirubin phenobarbital diazepam and oxazepam respectively Bilirubin was determined according to Michaëlsson et al (21) and phenobarbital according to Lous (16) Diazepam and oxazepam were determined by a gas chromatographic method Serum was extracted by benzene the extract evaporated and analysed in a gas chromatograph equipped with a ^{63}Ni detector (A Hansson and M Tonnesson unpublished) *These analyses were performed by A Hansson Ph D* Division of Toxicology Department of Clinical Chemistry Malmö General Hospital

RESULTS

Baseline VCO varied between 6 3 and 16 9 µmol/mmol TBH and day with a mean value of 12 6 and an S E M of 0 6 when all results were pooled (Table II) There were no significant variations between the three groups of baseline studies with a mean VCO of 13 0±0 8 in the phenobarbital group (n=10), 10 9±1 0 in the diazepam group (n=7) and 13 5±0 9 µmol/mmol TBH and day in the oxazepam group (n=7) As no significant differences were found when the material was divided into smokers and non smokers the subjects were pooled

The total variation for a single individual from period to period (three weeks) was 25% This includes the error of the method calculated as the coefficient of variation for the regression coefficient obtained in the determination of VCO This error ranged from 6 to 25% (mean 14%) After the administration of phenobarbital for seven days mean VCO was 15 1±1 8 µmol or 16% higher than in the baseline study The difference was not significant evaluated with the method of paired comparison (p>0 05) After seven days on diazepam VCO was 14 1±1 4 µmol/mmol TBH and day and after seven days on oxazepam 14 7±1 3 µmol/mmol TBH and day The increases correspond to 30 and 9% respectively of the baseline values but the differences are not significant However when the mean VCO for all the postdrug studies (14 7±1 0 µmol/mmol TBH and day) is compared with the mean VCO from all the baseline studies (12 6±0 6) the difference is probably significant (n=24 p<0 05)

Mean TBH calculated with the aid of CO dilution was found to be 52 5 51 7 and 52 2 mmol respectively in the three baseline studies (Table II) This corresponds to an average value of 11 7 g Hb/kg b wt Calculated from three baseline studies in each subject the variation in TBH from period to period in the same individual was 6% including the

Table II *Endogenous production of carbon monoxide (VCO μmol/mmol total body heme and day) serum b lirub n concentration (SB μg/ml) and total body heme (TBH mmol) before (B) and after (A) the ad m nistration for seven days of a dail dose of 100 mg phenobarb tal in the e ening (10 s bj) 15 mg d a epam divided into three daily doses (7 subj) and 75 mg oxa epam d vided into three da l d s (7 subj)*

Subj no	Phenobarb tal VCO B	A	SB B	A	TBH B	A	Diazepam VCO B	A	SB B	A	TBH B	A	Oxazepam VCO B	A	SB B	A	T B	A
1	9 1	13 8	? 1	3 4	51 3	43 9	6 9	14 1	? 3	1 8	5? 3	48 7	14.5	17 3	7 1	5 4		
?	1? 9	16 4	8 4	7 4	56 9	47 5	1? 1	6 9	9 9	7 1	5? 6	64 4	14 0	15 4	6 7	6 0	5	
3	10 9	18 3	14 0	10.2	50 4	45 3	13 8	17 1	1? 8	13 6	4? 7	44 3	16 9	13 8	11 4	11 4	50	
4	15 5	?8 9	4 3	4 9	45 4	43 9	7 6	13 6	? 4	3 0	40 4	46 6	11 9	8 3	? 4	3 9	4? 3	4
5	1? 8	11 ?	4 9	2 6	58.3	45 2	13.3	14 ?	4 6	5 4	48 6	37 8	15 ?	19 ?	5 0	4 0	5? 5	1
6	16 2	14 8	5 2	5 4	63 4	64 7	11 9	18 1	5 0	5 0	69 ?	65 3	11 0	14 ?	7 5	6 5	65 8	6
7	11 0	13 3	6 ?	6 ?	51 1	46 4	11 0	14.5	4 9	7 7	56 1	57 ?	11 0	14 ?	5 6	6 3	53 6	45
8	1? 8	11 0	4 9	3 4	57 7	56 7			–		–	–		–				
9	1? 4	9 0	4 1	2 3	37 7	38 7	–	–		–								
10	16 7	14 7	16 1	8 4	5? 6	51 3		–										
Mean	13 0	15 1	7 0	5 4	5?.5	48 4	10 9	14 1	6 0	6 ?	51 7	5? 0	13.5	14 7	6 5	6 ?	5?	4?
S D	?5	5 6	4 5	2 6	7 3	7 4	2 7	3 6	3 9	3 9	9 5	10 4	? 3	3 6	?8	5		9
S E	0 8	1 8	1 4	0 8	? 3	2 4	1 0	1 4	1 5	1 5	3 6	4 0	0 9	1 3	1 0	1 0		

error of the method After phenobarb tal TBH was 48 4+2 4 after diazepam 52 0±4 0 and after ox azepam 49 2±2 1 mmol The differences are not s gnificant ($p>0$ 05)

Mean baseline SB was 7 0 6 0 and 6 5 μg/ml respectively (Table II) The variation from period to period for a single individual was calculated to 16% After phenobarbital mean SB wa μg/ml and after diazepam and oxazep and 6 ?±1 0 μg/ml respect vel The d are not s gnificant ($p>0$ 05) In three nd two of whom had taken part in the studies de above 100 mg phenobarb tal were given b daily for seven days and VCO was stud ed on 0 2 4 and 7 The results are shown in Fig 1 No s gn ficant effect was found on VCO TBH r SB wh ch all stayed within the l mits of the n traindividual variation

The serum barbiturate concentration in the morn ing after seven days of phenobarb tal varied be tween 1? and 17 μg/ml (mean 15) The concentra tions of diazepam and oxazepam after seven days on the drug varied between 0 15 and 0 33 ng/ml (mean 0 25) and between 0 15 and 0 80 ng/ml (mean 0 45) respectively

Fig 1 Endogenous product on of carbon monoxide (VCO) serum b l rub n concentrat on (SB) and total body heme (TBH) in 3 healthy volunteers during the adm nistra t on of 100 mg phenobarbital (PB) daily in the evening for seven days The symbol with n parentheses ind cates a probably erroneous TBH determ nat on due to techn cal failure

DISCUSSION

The mean baseline VCO in our volunteers d d not differ significantly from values reported in the l ter ature for healthy adult males (3 6 9 17 18) Calcu lated in μmol/kg b wt and day our mean baseline VCO was 9 ?±1 0 compared to the figure of 8 9±0 6 μmol/kg and day given recently by Blaschke et al (3) The weekly variation and error

of the method are large but similar to the results of Lynch and Moede (18) which are the only data from groups large enough to give reliable results Since the error will be less if results are given as a fraction of TBH (that is in μmol CO/mmol TBH and day) because any error in the estimation of TBH will not then influence the determination of VCO we have preferred to use the relative values After phenobarbital for seven days no significant increase was found in VCO even though the relative mean rise was 16% The range was rather wide varying from an increase of 82% to a decrease of 27% Three individuals (nos 1 3 and 4) with increases of 66 68 and 82% respectively are responsible for the main part of the mean rise in VCO The significant increase in VCO after phenobarbital in these three individuals amounting to more than twice the intraindividual baseline variation in VCO from week to week might represent some genetically or environmentally determined aberration Excluding these three individuals mean VCO was unaffected, showing a non significant decrease of just 3%

Neither was any effect found for phenobarbital on TBH If anything there was a declining tendency with a mean decrease of 9% corresponding to 5 mmol heme or 80 g Hb This cannot be explained by blood letting alone since the maximum blood loss i two TBH determinations was 50 ml or 7 5

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Since animal experiments (23) have shown that the effect of phenobarbital on liver heme is rapid and that the liver heme pool might return to the baseline level within a few days three individuals were studied after only 2 and 4 days on the drug in *addition to baseline and post drug week studies* Even then no effect was found on VCO TBH or SB However all three individuals followed the same pattern (Fig 1) an increase on day 2 being followed by a decrease of 30% on day 4 and then a return to the baseline value on day 7 SB followed the same pattern except for the 2 samples drawn on day 7 At all events if phenobarbital does have an effect on total heme catabolism it must be slight and not measurable with the CO production technique

The same conclusions must be drawn from the diazepam and oxazepam studies These drugs did not appear to exert any significant effect on heme catabolism In the dog studies of Albanus et al (1) much higher doses were used which might explain the effect seen in enzyme inducing capacity especially with diazepam

That the drugs may have some effect on heme catabolism although small is suggested by the fact that when the results for all three were pooled a probably significant increase was found from mean baseline to mean postdrug VCO without any significant change in mean TBH

The heme-containing enzyme responsible for most of the hepatic heme turnover is P-450 which accounts for at least 40% of the heme content in rat liver (19) Due to its short half life this protein makes up at least 65% of hepatic heme turnover Studies in man are scant but recently Black et al (2) reported that the P-450 content was 12 nmol/g liver in biopsies taken during abdominal surgery In patients given phenobarbital 180 mg daily for seven days a decrease was found in those operated upon for hepatobiliary diseases compared to an increase of 30% in those undergoing surgery for other disorders Even though these results might explain part of the large interindividual variation seen in VCO after phenobarbital they lend no support to the assumption that phenobarbital in therapeutic doses increases the liver heme pool to such a degree that it boosts VCO by 70%

In a recent study published while the present manuscript was being prepared Blaschke et al (3) followed the effect of phenobarbital and glutethimide in therapeutic doses on hepatic bilirubin clearance plasma bilirubin turnover and VCO in 11 healthy volunteers They actually found a slight but non significant decrease in VCO after phenobarbital concomitant with a decrease in plasma bilirubin turnover the latter being responsi*ble for 30% of the decrease in bilirubin concentra*tion seen during administration of phenobarbital Apart from the three individuals with marked increases in VCO after phenobarbital the present findings are in close agreement with the results of Blaschke et al In a work from the same group Gisselbrecht and Berk (12) closely followed the total bilirubin production in rats before and during the administration of large doses of phenobarbital Even though they found increases of early labelled bilirubin varying between 200 and 300% and of liver P-450 of the same degree the total bilirubin production did not change suggesting that the effect of phenobarbital on total heme catabolism is slight

Our results should also be compared with

Coburn s data (5) wh ch prov ded the impetus for the present study Coburn found increases in VCO of 70% and in TBH of 10% after phenobarb tal given as in the present study and reach ng the same serum concentration We have no explanat on for the difference other than the fact that Coburn s material was small only 5 cases wh ch together w th the large ntra and interindiv dual variat ons in VCO m ght expla n the d screpancy

It has recently been reported (11 18 20) that VCO in fertile women is s gn f cantly h gher during the progesterone than the estrogen phase This in crease has been ascribed (11) to the enzyme nducing effect of progesterone (13) We have how ever been able to show (20) that the increase is most probably generated v a the bone marrow and/or the erythrocytes since it is accompan ed by a reticulocytos s Accord ngly although pheno-barb tal has a documented effect on l ver heme n the rat (23) this effect in man is apparently not large enough to be captured in terms of total heme catabol sm stud ed with the a d of VCO S nce l ver heme turnover represents only 15–20% of total heme turnover (19) and since the intra and in terind vidual variat on as well as the error of the method are large l ver heme turnover has to in crease at least three to fourfold to be captured This level does not seem to be reached with doses n the therapeut c range It is of l ttle value to make comparisons w th the effect of phenobarb tal on l ver heme in an mal experiments s nce the doses then g ven are usually 10–100 times larger (23) than n stud es in man

Agents exercis ng the r effect on the bone mar row and/or c rculating red cell heme pools are on the other hand more liable to be detected w th the CO product on techn que s nce these pools repre sent 80% of the heme turnover and a change of 30% or more w ll readily be detected

Even though it s reasonable to expect smoking to have some effect on the metabol sm of CO due to pers stent exposure to large amounts of this sub stance and an often tenfold increase n body CO pools such an effect was not found in the present study when the material was d v ded nto smokers and non smokers However the material is small and further stud es on th s subject are n progress

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Carbon Monoxide Production and Reticulocyte Count in Normal Women

Effect of Contraceptive Drugs and Smoking

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ABSTRACT Endogenous production of carbon monoxide (VCO), serum bilirubin (SB) and reticulocyte (retic) count have been followed during the menstrual cycle in 12 women (4 of them smokers) without contraceptive drugs and in 10 women (5 of them smokers) on contraceptive drugs. During the progesterone phase (Pph) VCO in the non smokers was 18 2±2 1 μmol/mmol total body heme (TBH) and day (mean ±1 S E M), compared with 10 1±1 0 during the estrogen phase (Eph) In the smokers VCO was 10 4±1 9 during Pph and 12,5±0 7 during Eph SB for the non-smokers was 5 2±1 0 μg/ml during Pph and 3 9±0 5 during Eph compared with 3 2±0 8 and 3 9±1 2 respectively, for smokers The retic count for the non-smokers was 64 1±4 7×10³/μl during Pph and 17 7±5 1 during Eph In the smokers the corresponding counts were 93 6±15 9 and 60 8±7 6 These results confirm earlier reports that VCO is increased during Pph and indicate that the rise could be due to a change in red cell catabolism since it is concomitant with a significant increase in retic counts which has not been found in earlier works on VCO, but which is known from reticulocyte studies Furthermore the results may indicate that persistent smoking affects the metabolism of CO and/or heme, since the smokers did not react with any change in VCO during Pph In subjects using contraceptive drugs, VCO was significantly higher (14 9±0 6 on combined estrogen-gestagen drugs and 14 5±1 1 on continuous gestagens respectively) than during Eph in non smokers (10 1±1 0 μmol/mmol TBH and day) which might be taken as the baseline group This indicates that the increased VCO in subjects on contraceptive drugs may be related to the activity of gestagens which were included in all contraceptive drugs tested

Recent reports indicate that hemoproteins of the liver may be responsible for a significant increase in endogenous production of carbon monoxide (VCO) after therapeutic doses of phenobarbital (5) and after caloric restriction (15) Furthermore a cyclical variation with an increase in VCO during progesterone phase (Pph) has been found in young female subjects (10 17) It has been suggested that the latter increase could likewise be due to an increased synthesis and turnover of liver heme proteins induced by progesterone (10) However since the reticulocyte (retic) count is increased during the late Pph immediately before the menstrual period (1) we thought it worthwhile to have a closer look at VCO during the menstrual cycle in young females with and without contraceptive drugs and relate the results to the retic count Pph was delineated with the aid of progesterone analyses Since there is no information on the effect of smoking on CO metabolism smokers were also included in the study

MATERIAL

Twenty two women aged 22-38 years (median 25) were studied Twelve of them (group A) had normal menstrual cycles and did not use any contraceptive drug eight were non smokers and had not smoked for at least one month (one subject) and usually not for at least three months four were smokers that is they regularly smoked 2-15 cigarettes daily The subjects in this group were studied three to six times during one menstrual cycle The other 10 women (group B) used some form of contraceptive drug Four (two smokers and two non-smokers) used a peroral gestagen (nor-ethisterone Mini P Astra Syntex AB Sodertalje Sweden) in a low (0 3 mg/day) continuous dose Two smokers used a long acting parenteral gestagen preparation (medroxiprogesterone Depo-Provera Upjohn & Co Kalamazoo USA) 150 mg i m every third month Finally three smokers and one non smoker used an estrogen-gestagen combination (0 5 mg norgestrel and

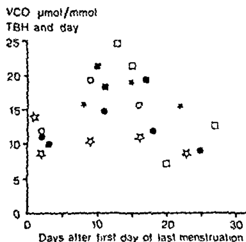


Fig 3 VCO related to time of menstrual cycle in 6 healthy young women using continuous gestagens for contraceptive purposes Two subjects were on 150 mg medroxyprogesterone i m every third month and four on 0 3 mg norethisterone daily by mouth □ ■=non smokers

Six women using gestagens only were studied four used a peroral and two a parenteral preparation two were non smokers VCO related to menstrual cycle in these women is plotted in Fig 4 Mean VCO in these six subjects was 14 5±1 1 µmol/mmol TBH and day When divided into smokers and non smokers the main finding was that the two non smokers showed large variations , a range from 7 2 to 24 6 µmol/mmol TBH and (8 studies in 2 subjects) while the four smokers .. less variation from week to week (Fig 4) with a range from 8 6 to 19 6 µmol/mmol TBH and day SB averaged 6 6±1 0 µg/ml and TBH expressed as g Hb/kg 9 2±0 3 g in these six cases

DISCUSSION

The present results confirm earlier observations (10 17) of an increased VCO during Pph VCO in our non smoking volunteers does not differ from the findings of Lynch and Moede (17) and Delivoria Papadopoulos et al (10) However we also found a simultaneous significant increase in the retic count Furthermore the increase in VCO was not demonstrable in our smoking subjects even though this group likewise had a significantly higher retic count during Pph than during Eph

The liver heme pool is very labile and is able to expand rapidly when exposed to enzyme inducing agents (22) So it is understandable that the increased VCO in Pph was attributed at first to the enzyme inducing effect of progesterone (12) However many facts seem to invalidate this hypothesis Firstly it was shown in 1951 by Berlin (1) that fertile women have a significantly higher retic count in the days immediately preceding the menstrual period Working with the Ashby technique Berlin also demonstrated a concomitant increase in red cell destruction We have been able to confirm these findings of Berlin—fertile women do have an increased retic count during Pph We are at a loss to explain why Delivoria-Papadopoulos et al (10) and Lynch and Moede (17) found no variations in the retic count in the women they studied Perhaps methodological differences are responsible We used a method for estimating the absolute number of reticulocytes which in our hands gives better accuracy and precision than methods where reticulocytes are counted as a percentage of red cells

VCO during Eph in non smoking women without contraceptive drugs may be taken as a baseline level and our mean value of 10 1±1 0 µmol/mmol TBH and day does not differ significantly from our normal value of 10 8±1 0 in healthy young males (15) Contraceptive drugs induced a significantly higher level 14 9±0 6 µmol in women on combined estrogen–gestagen preparations and 14 5±1 1 µmol in those on continuous gestagens The fact that higher values were found in both these subgroups suggests that it is the activity of gestagens which

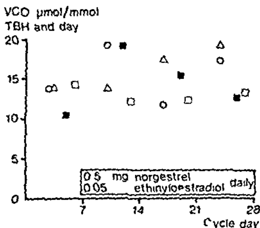


Fig 4 VCO related to time of menstrual cycle in 4 healthy young women one of whom was a smoker using a conventional estrogen-gestagen contraceptive drug daily for 21 days after a break of 7 days

causes the increase in VCO Progesterone may affect the red cell membrane making the cell more friable and more prone to destruction in vivo even though progesterone seems to increase red cell survival in vitro as demonstrated by Devenuto and Wilson (11) This hypothesis is supported by the recent finding of Oski et al (20) that the filterability of erythrocytes is decreased in women using a combined estrogen–gestagen contraceptive drug

If gestagens primarily affect red cells and increase their destruction then the increase in the retic count should follow and not be simultaneous with signs of increased heme catabolism Our data and those of Berlin (1) suggest that the reticulocytosis and increased heme catabolism are simultaneous Although the data are not sufficient to solve this problem they do not rule out the possibility that gestagens exert their effect not only on circulating red cells but also on the bone marrow Studies are in progress to correlate the filterability of erythrocytes to the retic count during the menstrual cycle

If it were true that the increase in VCO during Pph is due to changes in the turnover of liver heme proteins it has to be assumed that the liver heme pool increases three to five times This calculation is built upon the fact that the liver heme pool although small accounts for 20% of the daily heme turnover and that the increase in VCO during Pph amounts to between 65% in the present study and 100% in the work of Delivoria Papadopoulos et al (10) However data from the literature (3 9) do not support the idea that heme-containing enzymes in the liver show variations of this order of magnitude during the menstrual cycle or during the use of contraceptive drugs

Since persistent smoking may increase the body stores of CO by as much as ten times it is reasonable to assume that smoking may affect CO metabolism But no such effect has been demonstrated in man although Luomanmaki (16) found an increased oxidation of CO to CO in hypoxic dogs intoxicated with CO Recent studies on drug metabolism (19) have shown that many drugs have a shorter half life in smokers suggesting a larger pool of drug metabolizing probably heme-containing enzymes in the liver Even though this difference cannot be captured in the measurement of TBH with the CO dilution technique it might well explain the significantly higher VCO in the smokers during Eph If liver heme turnover represents 20% of the total daily heme catabolism an increase in total VCO by 25% would then involve an increase in liver heme turnover by 100% or more provided red cell turnover is unaffected It is conceivable however that the hypoxia induced by smoking has some effect on red cells and/or bone marrow and thereby also on heme catabolism of the erythron The fact that there was a tendency for the smokers to have higher retic counts than the non smokers during Eph as well as during Pph suggests that the difference in VCO between smokers and non smokers derives at least in part from an increased turnover of red cells in smokers

More remarkable is the fact that the smokers did not respond during Pph with an increase in VCO although their retic count increased to the same degree as the non smokers Another illustration of the difference is provided by pooling the results from the eight non smokers in group A and the two non-smokers on a low dose of continuous gestagens in group B (continuous gestagens may be said to be more physiological than estrogen–gestagen combinations) and comparing them with the pooled results from the five smokers in group A and the four smokers in group B using a continuous gestagen Mean VCO is then 13 3 and 12 4 μmol/mmol TBH and day respectively The S D from week to week within the same individual in the non smokers was 5 4 compared with 3 4 μmol in the smokers The difference is significant at the 1% level Accordingly the smokers had a more stable CO production than the non smokers whose VCO was probably significantly lower during Eph as well as significantly higher during Pph than the smokers Although the relative increase in retic count in the smokers was not the same as in the non smokers the fact that the smokers did show a significant rise during Pph might be interpreted as the assumed effect of gestagens on red cells even in the smokers It is not clear why this did not cause an increase in VCO Perhaps the increase in VCO during Pph is restricted to a short period only—between the eighth and third day before the menstrual period—and the observations in the smokers did not cover this period even though great care was taken to obtain data with the same intervals in both groups

It is also conceivable that the above mentioned effect of smoking on the liver pool of heme containing enzymes creates conditions for levelling out peaks in VCO by oxidizing CO into CO This

would not be detected with the method used in the present study since this measures only the net increase in the body CO pool

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Diurnal Variation in Endogenous Production of Carbon Monoxide

Effect of Caloric Restriction

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ABSTRACT The endogenous production of carbon monoxide (VCO) and total serum bilirubin (SB) have been followed in five healthy male volunteers during one baseline day and one day with no caloric intake. VCO in the morning studies was 11.2±1.7 (mean ±1 S.E.M.) on the baseline day and 10.1±2.3 μmol/mmol total body heme (TBH) and day on the fasting day, respectively. In studies before noon VCO increased significantly on both days, to values of 17.8±1.6 and 19.6±2.2 μmol/mmol TBH and day respectively. In the first study in the afternoon VCO differed significantly between the two days, amounting to 12.1±3.0 and 23.7±3.5 on the baseline and the fasting day, respectively. The difference was still significant in the evening when VCO was 11.6±3.1 and 22.1±4.9 μmol/mmol TBH and day. SB followed the same pattern, with mean values of 4.0±0.3, 4.9±0.3, 4.2±0.9 and 3.0±0.3 μg/ml during the baseline day and 4.5±0.6, 5.4+1.2, 7.0±0.5 and 8.5±1.0 μg/ml, respectively, during fasting day. Only insignificant amounts of conjugated bilirubin were found. The studies confirm earlier reports on the effect of caloric restriction on VCO. Since this effect is simultaneous with an increase in SB it is concluded that the changes are secondary to an increase in total heme catabolism. They might be due to an increase in intracellular hepatic heme turnover but it cannot be excluded that starvation affects erythropoiesis and/or red cell catabolism, thereby causing an increase in VCO and SB.

The mechanism behind the hyperbilirubinemia of fasting has been the subject of recent interest (2). It has been proposed that fasting induces a decreased hepatic bilirubin clearance (3). This finding might however be consistent also with the hypothesis of an increased intracellular hepatic bilirubin production temporarily blocking the uptake of the bilirubin from the blood (5). This hypothesis was based on the finding of a concomitant increase in the endogenous production of carbon monoxide (VCO) suggesting an increase in total heme catabolism. It has been known for a long time that serum bilirubin (SB) displays a diurnal variation, with higher levels in the morning (10). In 1971 it was shown that this increase can be suppressed by feeding during the night and that, on the other hand, SB increases as soon as five hours after the last meal (3).

To get more information on possible diurnal variations in heme catabolism, VCO was followed closely during the day in five healthy young men. The study was made twice: on one baseline day with a normal caloric intake and on one day without any caloric intake.

MATERIAL

Five healthy men aged 24–39 years (median 30) were studied four times during each of two days: from 8 a.m. to 10 a.m., from 11 a.m. to 1 p.m., from 2 p.m. to 4 p.m. and from 5 p.m. to 7 p.m. (studies 1, 2, 3 and 4). When the subjects arrived in the morning they had been fasting since 21.30 p.m. the day before. During the day of total caloric restriction, water was permitted ad libitum. During the day of normal caloric intake—baseline day—breakfast with coffee and sandwiches was served after the first study, lunch after the second study and coffee with snacks after the third study. The volunteers were allowed to walk about between studies but were told to avoid exertion and CO contamination. During the days before each of the studies they ate a normal diet. They denied abuse of alcohol or the use of any medication. They were all non smokers.

METHODS

VCO was determined by means of a closed rebreathing system described by Coburn et al. (4). For further details and calculations see Lundh et al. (6, 7). Total body heme

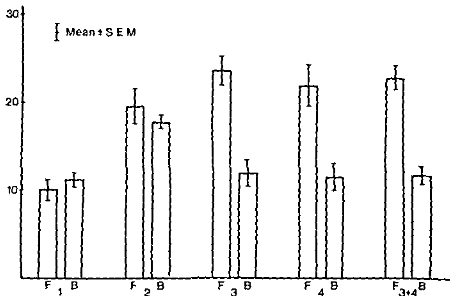


Fig 1 Diurnal variation of endogenous carbon monoxide production (VCO) in 5 healthy young men during one baseline (*B*) and one fasting (*F*) day *1* = 8 a m – 10 a m *2* = 11 a m – 1 p m *3* = 2 p m – 4 p m *4* = 5 p m – 7 p m *3+4* indicates that the results from studies 3 and 4 are pooled *TBH* = total body heme

(TBH) was determined only in the last study each day SB was determined according to Michaelsson et al (9) The bilirubin sample was drawn at the beginning of each study

RESULTS

Morning VCO in the total material varied between ¹ 4 and 15 2 µmol/mmol TBH and day with a mean ›e of 10 6 and an S E M of 1 3 There was no ˌ-rence between the two groups of morning dies with a mean VCO of 11 2±1 6 in the baseline study and a mean VCO of 10 1±2 3 µmol/mmol TBH and day in the morning of the fasting day The variation in VCO within a single individual from one day to the other was 28% This variation includes the error of the method expressed as the coefficient of variation for the regression coefficient obtained in the calculation of VCO (5) This error ranged between 8 and 40% (mean 20%)

During the baseline day mean VCO in studies 2 3 and 4 was 17 8±1 6 12 1±3 0 and 11 6±3 1 µmol/mmol TBH and day respectively The difference between studies 1 and 2 was significant while the decrease from study 2 to study 3 was not significant ($p<0\ 01$ and $p>0\ 05$ respectively) During the day of total caloric restriction mean VCO in studies 2 3 and 4 was 19 6±2 2 23±3 5 and 22 1±4 9 µmol/mmol TBH and day respectively The differences between the mean VCO for each of these studies and that of the morning study were all significant ($p<0\ 01$) Allthough the mean VCO for study 4 was slightly lower than for study 3 VCO in the evening was still about 120% higher than in the morning of the same day (Fig 1)

Comparing mean VCO between the two days showed no difference in study 2 In studies 3 and 4 the differences between the two days were probably significant ($p<0\ 05$) Pooling the results of studies 3 and 4 and comparing the baseline and fasting days gave a mean increase in VCO of 110% (range 46–182%) This increase was highly significant ($p<0\ 001$) (Fig 1)

On the baseline day SB decreased from 4 0±0 3 µg/ml in the morning to 3 0±0 3 in the evening ($p<0\ 05$) On the other hand SB increased significantly during the fasting day from 4 5±0 6 µg/ml in the morning to 8 5±1 0 µg/ml in the evening Evening SB on the fasting day was also significantly higher than on the baseline day ($p<0\ 01$) Pooling the results of studies 3 and 4 and comparing the baseline and fasting days showed that mean SB for the fasting day was 115% higher than for the baseline day ($p<0\ 01$) (Fig 2) Only insignificant amounts of conjugated bilirubin were found in the sera investigated

DISCUSSION

Morning VCO in the present study was identical to that reported earlier (3 4 6 7 8) The variation

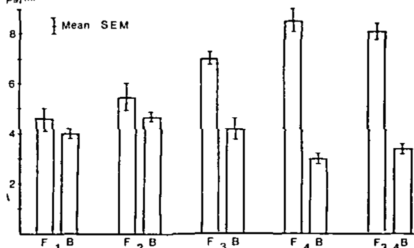


Fig 2 Diurnal variation in serum bilirubin in 5 healthy young men during one base line (*B*) and one fasting (*F*) day For explanation of the figures along the bottom see Fig 1

from day to day was large—on an average 20%—but of the same order of magnitude as reported by Lynch and Moede (8) who have studied this problem in detail The very high relative error of 40% had to do with the unusually low VCO of 1 4 µmol/mol TBH and day We have preferred to give results of VCO determinations in relative values (µmol/mmol TBH and day) in this report too even though it mainly concerns variations in VCO within the day from morning to evening

SB in our subjects showed the same diurnal variations as described earlier (2 3 10) The usual decrease during the baseline day was abolished by total caloric restriction and turned into a significant increase With this background we consider that the present data may be used to evaluate differences in heme catabolism between baseline and fasting days

During the fasting day mean VCO increased about 120% from the morning level thereby confirming earlier results on the effect of fasting on VCO (5 7) In the evening of the baseline day VCO was the same as in the morning The VCO of study 2 was increased on both days but there was no difference between the non fasting and fasting days It was not until study 3 after 16 hours of caloric deprivation that a difference appeared persisting in the evening (study 4) The simultaneous increases in VCO and SB strongly suggest that the changes are secondary to an increased heme catabolism However the data do not rule out the possibility that the findings are indirectly related to each other

The mechanism behind the effect of fasting on SB and VCO has been ascribed to an increased intrahepatic heme turnover secondary to induction of heme-containing enzymes (7) Other investigators have however reported a diminished hepatic bilirubin clearance during fasting (2 3) These two findings are not contradictory since an increased intrahepatic heme catabolism with formation of bilirubin and CO might block liver uptake of bilirubin from plasma Furthermore Bakken et al (1) have found in rats that the activity of heme oxygenase the enzyme converting heme into bilirubin and CO is increased in liver tissue during fasting

However it cannot be excluded that an increased turnover of hemoglobin heme is responsible for the changes in SB and VCO during fasting It is conceivable that caloric deprivation may affect erythropoiesis or that the lipolysis secondary to caloric deprivation may affect the red cell membrane changing its physical properties and making it more fragile

ACKNOWLEDGEMENTS

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The Di Guglielmo Syndrome A Study of 17 Cases

I Clinical and Haematological Manifestations

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ABSTRACT Based on a series of 17 patients with Di Guglielmo s syndrome i e , erythraemic myelosis and erythroleukaemia a description is given of the clinical and haematological manifestations of this disease Three cases were anerythraemic, i e no erythroblasts were seen in the blood smears, and three were aleukaemic i e no myeloblasts and/or promyelocytes were found in the blood The percentage of erythroblasts in the bone marrow smears varied from 34 to 96 In all cases abnormalities (often megaloblastoid changes) of varying severity were observed in the erythroblasts Proerythroblasts showing erythrophagocytosis, multinuclearity and vacuolation of the cytoplasm are features of great diagnostic importance Five patients had myeloblasts or promyelocytes containing Auer rods six had neutrophilic granulocytes showing pseudo-Pelger Huet anomaly, and in most cases agranular polymorphonuclears were present Brief comments are given on the PAS, nuclear arylsulphatase and naphtol AS D chloroacetate-esterase staining methods Four of five patients studied cytogenetically revealed abnormal stem lines The survival times after the onset of the disease ranged from 17 days to 20 months The disease is closely related to myeloblastic leukaemia In the course of Di Guglielmo s syndrome the leukaemic component often becomes more conspicuous, so that, in the terminal phase, the disease cannot be distinguished from typical myeloblastic leukaemia As, in Di Guglielmo s leukaemia' the diagnostic problems are greater than and different from those in typical myeloblastic leukaemia it is still reasonable to place this disease in a separate group under acute granulocytic leukaemia

In myeloblastic leukaemia and its variants such as promyelocytic and myelomonocytic leukaemias the erythropoiesis occasionally reveals sparse morphological changes of megaloblast like character This dysplasia is interpreted as evidence of involvement of the erythropoietic system in the neoplastic process When red cell precursors are prominently involved in the leukaemic process the disease is called Di Guglielmo s syndrome The disease to which Di Guglielmo s name was originally attached is the acute erythraemic myelosis (11 12) in which it is a characteristic feature that the neoplastic proliferation is confined to the erythropoiesis whereas both erythropoietic and granulopoietic hyperplasia occurs in erythroleukaemia Di Guglielmo s syndrome thus comprises both erythraemic myelosis and erythroleukaemia This terminology which is in agreement with that normally employed in the literature (3 9 10 11 12 13 16 22 23 44 62) will be used in this paper An erythraemic myelosis may be either acute or chronic (19) and it may be erythraemic or anerythraemic (with or without erythroblasts in the peripheral blood respectively) (3 5 12 22 23 42 45 46 53) An erythroleukaemia may likewise be erythraemic or anerythraemic (with or without myeloblasts in the peripheral blood respectively) (23)

While pure erythroblastic proliferation is rare (9 35 44 62) the more comprehensive group of Di Guglielmo s syndrome constitutes 8–13% of all *forms of acute leukaemias in adults (16 22)*

From Jan 1967 to May 1973 we encountered 17 patients with Di Guglielmo s syndrome in this hospital Nine of them were obscure cases of anaemia referred to us from other hospitals for further diagnostic investigation the disease had often presented as megaloblastic anaemia without response to vitamin B_{12} or folic acid therapy Based

Table I *Blood values and some cytological data at the time of diagnosis*

Case no	Age (y)	Sex	Hb (g/100 ml)	Nucleated cells (leucocytes+erythroblasts)/µl	Leucocytes/µl	Erythroblasts/µl	Myeloblasts and promyelocytes/µl	Reticulocytes	Platelets
1	42	♀	6 5	8 600	4 150	3 450	260	2 5	200
2	21	♀	4 5	2 500	2 250	250	100	0 6	70
3	71	♂	7 6	4 000	3 720	280	160	2–14	250
4	69	♂	8 3	3 500	3 500	0	0	0	40
5	81	♂	6 5	2 500	1 970	530	0	2 5–4	25
6	13	♂	7 7	2 400	1 560	840	190	2–19	35
7	81	♂	8 1	39 700	10 900	28 800	2 400	2	145
8	66	♀	9 6	29 300	26 370	2 930	2 630	2–6	60
9	62	♂	7 0	1 800	1 710	90	18	0–0 2	20
10	75	♀	7 5	1 700	1 700	0	0	0–0 6	20
11	44	♂	8 4	38 000	28 500	9 500	27 700	1–1 6	38
12	84	♀	6 9	2 500	2 500	0	25	0 2–1 6	140
13	80	♂	5 8	6 700	5 160	1 540	1 005	3–7	20
14	77	♀	6 7	4 200	3 070	1 130	168	2–6	120
15	76	♂	9 0	4 700	4 600	98	330	0 2	560
16	40	♂	7 0	2 800	2 320	476	448	0 7–5	35
17	76	♂	5 8	1 900	1 780	114	190	0–0 1	35

on this series the syndrome is discussed below in order to throw light on its clinical manifestations its relation to myeloblastic leukaemia and the diagnostic problems involved

METHODS

...gy of the blood and bone marrow cells was ...d in May Grunwald-Giemsa stained smears The ...e distribution of the bone marrow cells shown in Fig 1 is based on counts of 200–400 cells in oil immersion (×1 250) while that of the cells in the blood (Table I) is based on counts of 100–300 cells at the same magnification

Periodic acid-Schiff (PAS) staining and evaluation of the smears were performed by the methods described elsewhere (51)

Naphtol AS D-chloroacetate esterase staining

Solution A Naphtol AS D-chloroacetate 10 mg is dissolved in 1 ml N N-dimethyl formamide

Solution B One drop of 4% pararosaniline in 2 N HCl is mixed with one drop of 4% sodium nitrite in distilled water After 60 sec 30 ml 0 1 M Michaelis buffer pH 7 62 is added after which the pH is adjusted to 6 3 with 2 N HCl

The staining solution is prepared by mixing solutions A and B with subsequent filtration

Fixing fluid Amounts of 20 mg Na_2HPO_4 and 100 mg KH_2PO_4 are dissolved in 30 ml water 45 ml acetone and 25 ml formalin

Staining technique The smears are fixed for 30 sec *immersed in the staining solution for 30 min at room temperature rinsed in tap water for 3 min stained with* haematoxylin for 3 min rinsed in tap water for 10 min *immersed in distilled water and then mounted in Paragon®* mounting medium

MATERIAL

As appears from Fig 2 most of the 17 patients were adults of advanced age (average 62 2 years) Six were women (age range 21–84 years) and 11 men (age range 13–81 years)

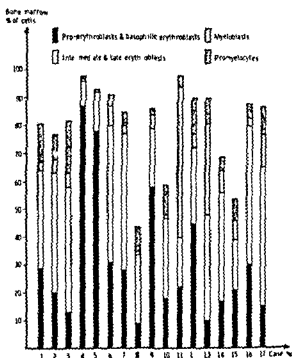


Fig 1 Cell distribution in the bone marrow

'ar ymorphs (% of blood ymorphs)	Abnormality in bone mar row erythro blasts (range 0–4)	Auer rods present	Pseudo-Pel ger Huet anomaly present
0	2–3	+	0
20	2–3	0	0
+55	1	+++	+
21	4	0	0
6	4	0	0
4	1–2	+++	0
10	3	0	0
36	1	0	0
'l	4	0	+
	2	0	+
50	1–2	0	0
17	2	0	+
16	1–2	+	0
6	1–2	0	+++
0	2	+++	0
8	1	0	0
+21	2–3	0	0

RESULTS

Clinical manifestations (Fig 3)

At the time of diagnosis the patients had had symptoms for ½–12 months (fig 4) As in other acute leukaemias they all complained of *fatigue* and were *pale* some had experienced *febrile episodes* and some had *lost weight* one patient (no 7) had had recurrent *joint pains* unaccompanied by gout In five patients (nos 1 2 12 14 and 17) *dyspepsia* in the form of nausea vomiting and sometimes pain (a feeling of burning or oppression) in the epigastric region had been a conspicuous symptom Only one *of the patients with dyspeptic discomforts revealed* palpable hepato and splenomegaly Relatively mild abdominal pain had been present in another four patients (nos 5 6 10 and 11) including one patient (no 11) with hepatomegaly *Bleedings into the skin* were observed in six patients (nos 5 6 8 10 13 and 17) *Enlargement of both the liver and spleen* was revealed in one patient (no 14) of the liver in two (nos 9 and 11) and of the spleen in two patients (nos 3 and 15) Four patients (nos 5 6 13 and 16) had mild to moderate *jaundice* Two patients (nos 5 and 17) revealed slightly enlarged lymph nodes on one side of the neck another patient (no 11) who suffered from pneumonia with sepsis had several slightly enlarged axillary and inguinal lymph nodes and in patient 6 the only child in the series small firm indolent cervical axillary and inguinal nodes were observed whereas no lymphadenopathy was seen in the remaining 13 patients One patient (no 1) complained of burning in the tongue and impaired *taste sensation for coffee*

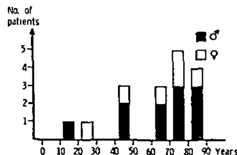


Fig 2 Age at the time of diagnosis

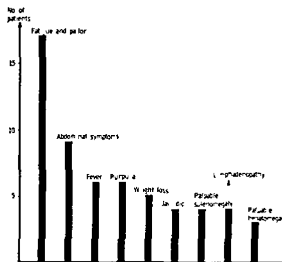


Fig 3 Clinical manifestations

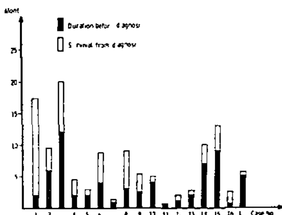


Fig 4 Duration of the disease

Blood

All the patients suffered from normochromic *anaemia* with Hb levels ranging from 4 5 to 9 0 g/100 ml (Table I) The anaemia was normocytic in 11 cases and slightly macrocytic in six The red cells in the peripheral blood showed anisocytosis in all cases Apart from the occurrence of erythroblasts *in the blood the most remarkable finding* was that six patients (nos 1 6 12 13 14 and 15) showed pronounced poikilocytosis with a large number of oval and drop-shaped erythrocytes—a poikilocytosis which was just as pronounced as that seen in myelofibrosis This phenomenon in Di Guglielmo s syndrome was also described by Linman (32) Cells with some degree of hypochromia occurred in five patients including four in *whom they were target cells Polychromasia and* slightly increased reticulocyte counts were often present We were under the impression that a certain relationship existed between the reticulocyte values and the number of erythroblasts in the peripheral blood—a correlation which was also observed by Baldini et al (3)

The total number of nucleated cells i e leucocytes plus erythroblasts in the blood varied from 1700 to 39700/μl *The fractions of the two types of* cells were calculated from the total count after dif [illegible] (Table I)

[illegible] 14 patients *erythroblasts in the blood* occur [illegible] in numbers ranging from 90 to 28800/μl at the time when Di Guglielmo s syndrome was diagnosed In two patients (nos 5 and 6) the number of erythroblasts in the blood increased from 530 to 30000 and from 840 to 21000/μl respectively *within observation periods of a few weeks* In most cases there was good agreement between the degree of the abnormality of the erythroblasts in the bone marrow and the peripheral blood In two patients (nos 3 and 8) the erythroblasts in the peripheral blood were normal while some of the bone marrow erythroblasts showed mild abnormalities Three patients (nos 4 10 and 12) were anerythraemic One of them (no 4) has been described previously (53)

The leucocyte counts varied from 1700 to 28500/μl Three patients (nos 4 5 and 10) were aleukaemic at the time of diagnosis Incidentally the number of myeloblasts plus promyelocytes ranged from 18 to 27700/μl The peripheral blood often contained monocytoid cells of an immature appearance (cells belonging to the reticuloendothelial system abnormal promyelocytes without granules?)

In the majority of cases *agranular polymorphonuclears* were present in the blood and bone marrow smears Thus in the Giemsa stained blood smears 4–55% of the segmented cells did not contain granules (Table I)

The pseudo-Pelger Huët nuclear anomaly was seen in a few of the neutrophilic polymorphonuclears in six of the 17 patients This was most pronounced in patient 14 in whom 30% of these cells showed this anomaly

As we beforehand were under the impression that erythroleukaemia—just like other myeloproliferative disorders—may reveal an increase in the number of eosinophilic cells this was assessed *both in the blood and bone marrow smears* The percentage of eosinophilic cells varied from 0 to 5 in the bone marrow and from 0 to 11 in the peripheral blood but only in one patient (no 6) was the total count of these cells above normal The bone marrow often revealed scattered immature eosinophilic cells with markedly vacuolated cytoplasm and sometimes an uneven distribution of granules

As *appears from Table I the platelet counts* varied within fairly wide limits at the time of diagnosis and were perhaps on the whole a little higher than in other acute leukaemias The platelet counts were 200000/μl or more in three patients and below 50000/μl in nine

Bone marrow

The bone marrow was studied in smears sections *of the clot and in some cases in biopsy specimens* from the iliac crest In patient 14 in whom it was difficult to obtain cellular smears the section of the bone marrow clot showed maximum hyperplasia In keeping with the definition of the syndrome all the patients revealed pronounced *hyperplasia of the erythropoiesis* the percentages of erythroblasts varying from 34 to 96 (Fig 1) In addition they all exhibited morphological anomalies (i e *dysplasia*) *of varying severity in the erythropoiesis* An attempt was made to illustrate the degree of abnormality by grading the cases from 0 to 4 (Table I) In the cases with the mildest bone marrow anomalies (grades 1–2) only slight changes were present in a few cells taking the form of a slightly abundant amount of cytoplasm nuclear constrictions a distinct nuclear chromatin pattern and dissociation be



Fig 5 Proerythroblasts with marked budding and vacuolation of the cytoplasm 1 770 (Patient 5)

Fig 6 Immature erythroblasts Note the distinct chromatin pattern in the cell to the right × 1 770 (Patient 9)

Fig 7 An erythroblast and a myeloblast the latter containing an Auer rod from a patient with erythroleukaemia × 1 770 (Patient 15)

Fig 8 Naphtol AS D-chloroacetate-esterase staining two neutrophilic polymorphonuclears one with grossly irregular distribution of the positivity the other agranular showing no staining at all × 2 796 (Patient 15)

tween the degrees of maturation of the nucleus and cytoplasm In the most abnormal cases (grades 3–4) the cytological picture was highly polymorphic with a pronounced shift to the left as evidence of maturation arrest (i e *anaplasia*) sometimes characterized by giant cells multinuclearity and multilobularity and many mitoses were present In a few cases the nuclei contained large nucleoli The basophilic erythroblasts and the proerythroblasts very often exhibited pronounced cytoplasmic budding and constrictions of some cytoplasm and the cytoplasm was markedly vacuolated (Fig 5 pat 5) The patient concerned was referred to us with a tentative diagnosis of myelomatosis All the patients revealed multinucleated cells i e binucleated and often trinucleated erythroblasts Occasionally the number of nuclei or lobules was appreciably higher thus in patient 4 up to 10–12 lobules were observed In many cases the erythroblasts were similar to the megaloblasts seen in vitamin B_{12} and folate deficiency (Fig 6) In eight of the patients (nos 4 5 6 7 9 10 13 and 16) erythroblasts which had phagocytized erythrocytes were observed (49) Patients with a preponderance of intermediate and late erythroblasts (nos 3 10 and 15) often ran a less acute course than those in whom proerythroblasts and basophilic erythroblasts predominated (Figs 1 and 4)

The granulopoiesis in most cases showed a shift to the left with a slightly to moderately increased number of myeloblasts and promyelocytes (Fig 1) which often had large nucleoli and abnormally coarse granules Thus these patients had both erythropoietic and granulopoietic hyperplasia (i e erythroleukaemia) Three patients (nos 5 7 and 16) revealed blasts of a morphology which made it difficult to decide whether they were myeloblasts or primitive erythroblasts Other authors (16) have called attention to the same observation The smears were carefully studied for the presence of *Auer rods* (Fig 7) in the myeloblasts and promyelocytes Auer rods were found in five patients fairly numerous in patients 3 6 and 15 whereas we observed this inclusion body in a single cell in the remaining two cases Two of the patients with many Auer rods belonged to the group with the longest survival times (Fig 4)

Distinct megaloblastic dysplasia in the granulopoiesis with the occurrence of giant juveniles like those seen in vitamin B_{12} and folate deficiency was encountered in one patient (no 12) In this patient who was the oldest in the series the erythroleukaemia was complicated by folate deficiency (54) A few relatively large juveniles in the bone marrow and some macropolycytes with 6–7 nuclear segments were observed in patient 8 and such hypersegmented cells were also seen in patients 2 4 6 and 8 Two of the latter possibly suffered from folate deficiency as suggested by a severely pathological Figlu test (pat 4) and serum levels of folate at the lower limit of the normal range (pat 6) In the remaining two patients the presence of macropolycytes could presumably be ascribed to folate deficiency locally in the bone marrow

In the bone marrow smears a common feature was the presence of only a very few megakaryocytes the cells of this type were often hypersegmented and without platelet formation

The bone marrow smears from three patients (nos 3 8 and 15) revealed plasma cells of the flaming cell type In patient 3 an immunoelectrophoretic study of the serum early in the course had aroused suspicion of incipient IgG paraproteinaemia—a finding which could not be confirmed in a repeat study performed a few months later In the other two patients (nos 8 and 15) immunoelectrophoresis of the serum did not suggest the presence of a paraprotein

In five patients *cytogenetic studies of the bone marrow cells* were performed (by Dr M Krogh Jensen) Patients 6 10 12 and 16 had abnormal stem lines with 47 59 44 and 45 chromosomes respectively and blurred chromatin which is strongly suggestive of acute leukaemia (24 25) Patient 3 had a normal chromosome number but blurred chromatin

Cytochemical studies

PAS staining of the bone marrow smears showed that many but not all the patients had strongly PAS positive erythroblasts (51)

Naphtol AS D chloroacetate esterase staining of blood and bone marrow smears was performed in the 12 patients in whom the disease was diagnosed in the last part of the investigation period This enzyme is attached to neutrophilic granules On staining by this method the granules in the promyelocytes and myelocytes and Auer rods if any become even more conspicuous than in Giemsa stained smears (1 20 27 28 29 56) As already stated Auer rods were found in five cases In sev

eral cases the agranular polymorphonuclears mentioned in the section on Giemsa staining became very distinct in the esterase stained smears (Fig 8) while other neutrophilic granulocytes from these patients had a very grossly irregular distribution of the esterase activity (Fig 8) In naphtol AS D-chloroacetate-esterase staining neither the pathological erythroblasts in Di Guglielmo s syndrome nor the megaloblasts in vitamin B_{12} and folate deficiency take any stain (30 56)

Nuclear arylsulphatase activity was assessed in some of the patients (52) Positive nuclear activity of varying intensity was observed in the erythroblasts both in Di Guglielmo s syndrome and in vitamin B_{12} and folate deficiency which implies that this method is not of value in the differentiation

DISCUSSION

The diagnosis of Di Guglielmo s syndrome is based on the clinical findings and an evaluation of the morphology of the cells in the blood and bone marrow We hold that the best possibilities of definitely establishing the diagnosis exist when the clinician personally is fully familiar with the bone marrow cytology so that he can correlate the abnormal blood and bone marrow findings with the clinical and the laboratory data

clinical symptoms are identical with those in myeloblastic leukaemia fatigue anaemia ι loss and in some cases bleedings into the skin febrile episodes hepatosplenomegaly jaundice and rarely enlargement of lymph nodes

Some of our patients presented a variety of uncharacteristic abdominal symptoms similar to those observed in some cases by Sheets et al (46) and Van Hove and Hamers (21) There may be a causal relationship between those complaints and growth of ectopic bone marrow in the liver and spleen

On the basis of the series considered in this paper nothing definite can be said of the incidence of Di Guglielmo s syndrome within a certain geographical area or of the frequency of this leukaemia as compared with other forms of acute leukaemia This is due to the fact that in these patients the diagnosis is usually attended with great difficulties Accordingly they are often referred from other hospitals to a special department of haematological medicine while a number of patients with clear-cut leukaemias from the same area are referred to the radium centres

Some important factors in the diagnosis of Di Guglielmo s syndrome are summarized below

1 *The presence of a hyperplastic erythropoiesis usually revealing megaloblastic changes* All our patients exhibited dysplasia of the erythropoiesis (Table I) In some cases, the changes in the red cell precursors are so slight that they may be overlooked by the less experienced examiner or the erythropoiesis in Di Guglielmo s syndrome may be normoblastic (8 39) Accordingly the possibility of this diagnosis should not at once be excluded—as we have seen in some cases—just because pronounced changes in the erythropoiesis are not present However there are certain abnormalities in the erythropoiesis to which we attach particular importance such as the demonstration of proerythroblasts which have phagocytized erythrocytes (50) the presence of many multinuclear erythroblasts and markedly vacuolated cytoplasm in the proerythroblasts and basophilic erythroblasts Almost invariably we found highly immature erythroblasts with distinct cytoplasmic budding and constrictions From the description given by Hayhoe et al (17) it might be thought that cytological budding justifies a suspicion of Di Guglielmo s syndrome However in our opinion this is not the case as in our experience similar proerythroblasts are often present in other patients with pronounced erythropoietic hyperplasia (vitamin B_{12} deficiency autoimmune haemolytic anaemia hereditary spherocytosis) Reardon and Tanaka (41) expressed the view that cytoplasmic buds are in particular characteristic of cells of lymphoid origin As just stated we have not infrequently seen this phenomenon in erythroblasts

The morphology of the bone marrow erythroblasts in Di Guglielmo s syndrome may be similar to that seen in HEMPAS (Hereditary Erythroblastic Multinuclearity with a Positive Acidified Serum Test) (7) Although only a few of our patients were subjected to the acidified serum lysis (Ham) test we find however that any suspicion of this hereditary disease can be ruled out Most of our patients were adults of advanced age with a short duration of haematological symptoms and as mentioned before the patients often showed disturbances in the granulopoiesis and thrombopoiesis and/or had other signs or symptoms indicating leukaemia In HEMPAS there is no evidence that leucocytes and platelets are affected (7)

2 *The presence of megaloblastic changes in the*

erythropoiesis without a similar dysplasia in the granulopoiesis provided that the disease as in our patient 12 is not complicated by folate deficiency

3 *It is important to exclude vitamin B_{12} and folate deficiency as the cause of the pathological erythropoiesis* This may be done by administering these vitamins for a short period

4 Even though erythroblasts and myeloblasts plus promyelocytes most frequently occur in the blood the disease may be *anerythraemic* and/or *aleukaemic* Three of our patients were anerythraemic and three were aleukaemic at the time of diagnosis We have seen others reject a diagnosis of Di Guglielmo s syndrome either because there were no erythroblasts in the peripheral blood or because it was found that these cells were not present in a sufficient number

5 *Strong PAS positivity of the erythroblasts lends support to the diagnosis* (1 3 15 17 23 33 34 40 47 51) but low PAS scores or even one of zero do not exclude the diagnosis (23 43 51 63) PAS scores of 40 or more for proerythroblasts plus basophilic erythroblasts and intermediate plus late erythroblasts were observed in 12 and 13 respectively of our 17 patients (51)

6 In cases in which there is also granulopoietic hyperplasia the demonstration of *Auer rods in myeloblasts and/or promyelocytes* is strongly in favour of a diagnosis of leukaemia since these inclusion bodies have so far been described only in acute myeloblastic leukaemia and its variants (4 17 61) However referring to a case of possible leukemoid reaction in disseminated tuberculosis with Auer rods in the cells described by Leavell and Twomey (26) Wintrobe (62) writes that it remains to be proved that their formation is a phenomenon limited to leukemia We found these abnormal inclusions in bone marrow smears from five of the 17 patients although they were present in many of the cells only in three (nos 3 6 and 15) In two of these patients with many Auer rods (nos 3 and 15) the disease ran a subacute course (Fig 4) Other authors (20) have reported that the presence of Auer rods may be related to relatively long survival

7 The demonstration of *cytogenetic abnormalities* in bone marrow cells with the occurrence of abnormal stem lines strongly favours a diagnosis of leukaemia As in other forms of acute leukaemia such changes presumably occur in about one half of the patients (18 24 25) We found abnormal stem lines in four of five patients who were studied for this abnormality

8 All cases of Di Guglielmo s syndrome so far described have run a *fatal course* whether they were treated or not This fact may also be taken as an important characteristic of the disease

9 *Autopsy* usually reveals severe *bone marrow changes* and sometimes *widespread infiltration in various organs* (55)

In the study of the morphology of blood and bone marrow cells the following observations may be of diagnostic significance

10 The occurrence of *agranular polymorphonuclears* as such cells are often encountered in acute myeloblastic leukaemia and its variants (17) We found that a varying percentage of the polymorphonuclears was without granules in 15 of our 17 patients (Table I) Such defective cells could also be demonstrated in naphtol AS-D-chloroacetate-esterase stained smears Similar observations were made in Di Guglielmo s syndrome by Huhn et al (22) and in acute myeloblastic leukaemia and erythraemic myelosis by Leder (27 28 29) whereas Catovsky et al (6) demonstrated a peroxidase defect in the neutrophilic granulocytes from patients with acute leukaemia of the myeloid type However it has apparently not been sufficiently studied how often such agranular polymorphonuclears are present in other disorders of the blood and in control series of normal subjects

11 The occurrence of neutrophilic polymorphonuclears with the *pseudo-Pelger Huet nuclear anomaly* which has previously been described in leukaemia (2 4 17 22 27 28 61) We found such cells in six of our 17 patients This anomaly has also been observed by other authors in association with erythroleukaemia (38 48 49) The case reported by Phillips et al (38) is stated to be the first in which this cellular anomaly was observed in a patient of whom it was definitely known that normal neutrophilic granulocytes had been present before the diagnosis of leukaemia

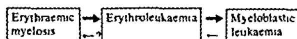
In many of our cases we found a certain degree of monocytosis in the peripheral blood which is in agreement with the observations reported by Dameshek and Gunz (9) and Schwartz and Critchlow (44) These monocytoid cells are presumably a component in the proliferation of the reticuloendothelial system which others have demonstrated in the bone marrow in Di Guglielmo s syndrome (4 9 21 39) In our experience a similar

monocytosis is often present in other myeloproliferative disorders such as polycythaemia vera and myelofibrosis

We found plasma cells of the flaming cell type in three of the patients in the series Reports in the literature (14 37 59) suggest that this type of plasma cells occurs only in myelomatosis and in particular in the IgA producing myeloma We do not hold with this assertion as we have observed flaming cells in diseases other than Di Guglielmo s syndrome (57) The explanation of the discrepancy may be that our flaming cells were less flaming than those described by Waldenström (59) The flaming cells in our patients were similar to those illustrated by Paraskevas et al (37) in their Figs 2 *and 6*

Even if we try to segregate Di Guglielmo s syndrome as a special group under acute granulocytic leukaemias there is much evidence suggesting that erythraemic myelosis and erythroleukaemia are myeloproliferative disorders closely related to myeloblastic leukaemia Hb abnormalities have been demonstrated both in Di Guglielmo s syndrome (36 40) and in typical acute myeloblastic leukaemia (60) This together with the fact that transitional forms between these two disorders exist favours the assumption that the individual types different stages of one and the same dis In some patients with Di Guglielmo s syn me the leukaemic component gradually be more conspicuous while the red cell precursors simultaneously recede into the background to such an extent that in the terminal phase the disease cannot be distinguished from typical *myeloblastic leukaemia* (3 8 9 16 21 45) *Like Dameshek and Gunz* (9) we have seen cases in which an initially pure erythroblastic proliferation within weeks or months through myeloblastic *proliferation reached the stage of a mixed cell population* (erythroleukaemia) and occasionally ended in a typical acute myeloblastic leukaemia while cases diagnosed as erythroleukaemia only had to pass through the last of these stages Dameshek and Gunz (9) expressed the view that about half of the primary erythraemic cases will pass through these stages The probability of this change in the leukaemic direction presumably increases with the length of survival (3 58) The reverse course i e from a typical myeloblastic leukaemia to erythroleukaemia has also been observed in rare cases We have had an opportunity to observe such a case but we have never seen a transformation from erythroleukaemia to pure erythraemic myelosis The latter possibility must be queried although both Dameshek and Gunz (9) and Lien Keng (31) report having encountered it

This tendency to transitions between the individual cytological forms was aptly illustrated by Pribilla (39) in the following diagram



The disease may end in any of the three stages

The fact that a case of acute leukaemia may occasionally be classified either as erythroleukaemia or myeloblastic leukaemia with a conspicuous erythraemic component also provides evidence in support of the existence of sliding transitions between the two types Our patients 11 and 13 are examples of this

As it is thus a characteristic feature in Di Guglielmo s syndrome that many of the cases in time change cytologically into a typical acute myeloblastic leukaemia we find—in agreement with some other authors (3 8 9 10 11 12 13 21 39 45)—that this change does not justify their exclusion from the group—on the contrary it is a contributory factor serving to confirm the diagnosis

The question may then well be asked Is any purpose served by segregating Di Guglielmo s *syndrome* as a *special group within* acute granulocytic leukaemia? As far as the aetiology pathogenesis and treatment are concerned it is reasonable to consider the two diseases together *but when we deal with the diagnostic problems* which—as already mentioned—are often considerable and of a different nature in this leukaemia it seems reasonable to keep this variant in a separate group Accordingly we can fully endorse Dameshek s words (8) We believe therefore there is sufficient reason to warrant the continuing use of the designation Di Guglielmo s syndrome

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The Di Guglielmo Syndrome A study of 17 Cases

II *Periodic Acid Schiff Staining of the Erythroblasts*

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ABSTRACT In 17 patients with Di Guglielmo s syndrome the PAS-stained erythroblasts in bone marrow smears have been assessed by a scoring system Most of the PAS scores were either moderately high or very high—much higher than those normally seen in disorders with benign erythropoietic hyperplasia On the other hand, very low scores were obtained in a few cases Whereas a high PAS score always lends strong support to a diagnosis of Di Guglielmo s syndrome a low score, or even a negative reaction does not exclude this diagnosis The highest scores were obtained in cases with many pathological erythroblasts Staining after diastase digestion confirmed that the positive PAS reaction is due to glycogen In this test, experience showed that a period of at least 2 hours is required for complete digestion of the glycogen

In Di Guglielmo s syndrome the erythroblasts are often pathologically changed In some cases these cells show a confusing similarity to the megaloblasts in pernicious anaemia and folate deficiency In other cases the erythroblasts are so immature and abnormal that they may give rise to difficulties in the differentiation from other cells e g myeloma cells In view of these problems we have studied the value of periodic acid Schiff (PAS) staining in the diagnosis of Di Guglielmo s syndrome

MATERIAL AND METHODS

The series studied consisted of 17 patients with Di Guglielmo s syndrome in whom the diagnosis had been established on the basis of clinical signs and the cell morphology as assessed in Giemsa stained blood and bone marrow smears The designation Di Guglielmo s syndrome here includes both erythraemic myelosis and erythroleukaemia i e leukaemia cases in which red cell precursors are either highly predominant or constitute an appreciable component in a mixed myeloid leukaemia The clinical and haematological data from the patients studied are reported in detail elsewhere (27)

In these patients bone marrow smears were subjected to PAS staining in order to assess the erythroblasts This was done in all patients before the institution of treatment and in 11 it was repeated later in the course of the disease as follows in patients 4 5 10 13 and 17 once in patients 6 and 8 from five to nine times

The smears from 13 of the patients were subjected to PAS staining within the first week after aspiration of the samples while in the remaining four (nos 1 2 5 and 17) the staining was performed on Giemsa stained smears which had been mounted in Canada balsam with xylol and stored for 1–5 ½ years Quaglino and Hayhoe (21) pointed out that the study of such PAS stained smears is of value even if they have been stored for several years

For the purpose of comparison PAS staining was performed on erythroblasts from such other diseases as pernicious anaemia folate deficiency and haemolytic anaemias and on plasma cells in myelomatosis

Staining technique

The preparations used in this study were invariably subjected to Giemsa staining before PAS staining The PAS staining consisted of the following steps oxidation with 0 5% periodic acid for 10 min rinsing in water for 1–2 min staining with Schiff's reagent for 20 min at room temperature washing in tap water for 10 min in order to remove excessive dye dehydration in increasing concentrations of ethanol (70 76 and 99%) and then in xylol The preparations were finally mounted in Eukitt*

In order to check the PAS staining technique simultaneous staining was performed on smears with normal neutrophilic granulocytes which are positive

Prior to the PAS staining some smears were subjected to *diastase digestion* as described by Dacie and Lewis (4) and Hayhoe et al (12) in order to disclose if the PAS positivity was due to glycogen If a positive reaction is due to glycogen it disappears after such treatment while other PAS positive material is unchanged (4)

Table I *PAS scores for the most immature erythroblasts*

Case No.	PAS score for 100 consecutive erythroblasts — Distribution of positivity 0	1	2	3	4	Total score
1	28	15	25	27	5	166
2	53	31	11	5	0	68
3	61	38	1	0	0	40
4	22	10	10	27	31	235
5	2	9	29	52	8	255
6	39	32	15	13	1	105
7	26	6	35	29	4	179
8	57	17	11	15	0	84
9	1	3	4	11	82	372
10	24	42	24	10	0	120
11	62	24	14	0	0	52
12	64	30	6	1	0	45
13	71	23	6	0	0	35
14	57	15	10	13	5	94
15	76	21	3	0	0	27
16	98	2	0	0	0	2
17	75	17	8	0	0	33

Films for diastase digestion were exposed to a diastase solution (0 1 g/100 ml 0 9% NaCl) at room temperature for 1 hour as recommended by Dacie and Lewis (4) and also for 2 hours as proposed by Skjoldborg (25) and then stained as described above The diastase-PAS technique was performed on preparations from three of the patients (nos 4 8 and 9) in whom the erythroblasts showed the strongest PAS positivity (Tables I and II)

The PAS positivity of the erythroblasts was assessed in 100 consecutive cells studied in oil immersion (×1 250) and scored by roughly the same method as described by ayhoe et al (12)

tings for proerythroblasts and basophilic erythroblasts

0 Negative cytoplasm devoid of granules 1 Fine granules present 2 Moderately coarse granules present 3 Coarse granules present 4 A block or blocks present

Ratings for intermediate and late erythroblasts

0 Negative 1 A pale reddish tinge in the cytoplasm 2 A moderately deep diffuse red tinge in some cases also numerous scattered granules 3 An intense diffuse red staining in the cytoplasm in some cases also very coarse granules

The sum of 100 consecutive ratings provided the score for the individual specimen Illustrative examples of the erythroblast ratings are shown in Figs 1-6

The PAS scoring of erythroblasts according to the above mentioned principle was based exclusively on the judgement of the author who at the time of the study was fully cognizant of both the clinical manifestations and the cytological findings in the Giemsa stained smears

RESULTS

The assessment of the PAS stained smears resulted in the scores shown in Tables I and II These scores were based on an evaluation of just one smear before the initiation of treatment Many of the patients had strongly PAS positive erythroblasts but there were also a few with very low scores Tiny fragments torn off from the cytoplasm were also often intensely PAS positive (Fig 5) In proerythroblasts and basophilic erythroblasts the positivity was seen in the form of fine or coarse granules or clumps against an unstained background cytoplasm while the intermediate and late erythroblasts yielded a diffuse cytoplasmic PAS staining varying in tinge from faint pink to deep red In the latter type of cells the diffuse red tinge was associated with granular PAS positivity in some cases The smears with strongly PAS positive erythroblasts in patients 1 2 and 5 had been stored for 5½ 4 and 1½ years respectively before this staining In most cases the same patient showed PAS negative erythroblasts side by side with intensely PAS positive erythroblasts (Tables I and II) The percentage of PAS positive cells ranged from 2 to 99 for both proerythroblasts plus basophilic erythroblasts and intermediate plus late erythroblasts

In some patients (e g nos 5 and 9) both proerythroblasts plus basophilic erythroblasts and intermediate plus late erythroblasts showed strong PAS positivity while in others a particularly intensely positive reaction was observed either in the most immature (e g patient 4 (Fig 4) see also Tables I and II) or in the more mature erythroblasts (e g patient 8 Fig 3 Tables I and II)

Table II *PAS scores for the more mature erythroblasts*

Case No	PAS score for 100 consecutive erythroblasts — Distribution of positivity 0	1	2	3	Total score
1	12	56	22	10	130
2	46	49	4	1	60
3	42	54	4	0	62
4	55	20	8	17	87
5	1	17	49	33	214
6	88	10	2	0	14
7	40	39	16	5	86
8	6	25	28	41	204
9	25	12	8	55	193
10	28	35	16	21	130
11	75	20	4	1	31
12	59	26	14	1	57
13	94	4	2	0	8
14	47	37	11	5	74
15	89	10	1	0	12
16	98	2	0	0	2
17	66	29	4	1	40

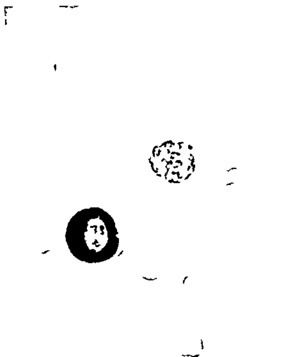


Fig 1 PAS positive (ratings 1 2 and 3) intermediate erythroblasts ×1215 (Patient 9)

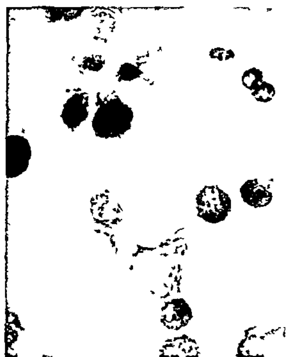


Fig 3 PAS posit ve (rating 2) lobulated late erythroblast (top) PAS pos tivie (rating 1) trinucleated intermed ate erythroblast (bottom) and some basophil c erythroblasts with granular positivity (ratings 1 ard 3) ×1215 (Patient 8)



Fig 2 PAS react on diffuse positivity (rat ng 2) in a giant mult nucleated intermed ate erythroblast and some basoph l c erythroblasts with f ne (rat ng 1) or coarse granules (rating 3) ×1215 (Patient 4)



Fig 4 PAS positive proerythroblasts and basophil c erythroblasts w th moderately coarse (rating 2) or coarse granules (rating 3) or blocks (rat ng 4) of PAS-positive material in the cytoplasm ×1215 (Patient 4)



Fig 5 Intensely PAS positive (rating 4) proerythroblasts showing distinct budding and fragmentation of the cytoplasm ×1215 (Patient 9)

Generally the morphologically most abnormal proerythroblasts and basophilic erythroblasts showed the most intense PAS positivity (Fig 7) Observations made in the course of the disease in patient 15 may support such a relation to the cell morphology At the time of diagnosis and for a uple of months while he was under observation thout treatment this patient revealed moderate egaloblastoid features in the erythropoiesis and had low PAS scores After the first course of treatment with cytostatic drugs the bone marrow cytology changed radically Highly immature erythroblasts now became predominant and the cells were severely abnormal showing marked vacuolation of the cytoplasm multinuclearity and large nucleoli Morphologically the cells resembled the abnormal erythroblasts seen throughout the course in patient 9 whose erythroblasts showed the most intense PAS positivity The change in cell morphology in patient 15 was accompanied by a steep increase in the PAS score viz for proerythroblasts and basophilic erythroblasts from 27 and 56 at two examinations carried out at an interval of $2^1/_2$ months before the institution of cytostatic therapy to 226 after this therapy

Repeated PAS staining of smears from one and the same patient showed that the PAS reaction usually remained at roughly the same level Thus in patients 4 5 8 and 9 intense PAS positivity of the erythroblasts persisted This should be viewed in relation to the clinical course none of these patients obtained complete remission Erythroblasts with moderately high (pat 10) or low PAS scores (pats 3 13 16 and 17) usually also remained unchanged The change which occurred in the terminal phase in patient 15 is mentioned above

In patient 6 in whom two evaluations were performed at an interval of 4 weeks before treatment some increase in the PAS score occurred viz from 105 to 175 for the proerythroblasts plus basophilic erythroblasts and from 14 to 25 for the intermediate plus late erythroblasts This patient was the only one in whom a brief partial haematological remission was obtained In relation to this remission PAS scores of 21 and 4 respectively were found for the two groups of erythroblasts A few weeks later the patient had a relapse with predominance of myeloblasts (60–80%) while initially he had shown a distinct preponderance of erythroblasts (80%) Even in the terminal phase this patient had PAS scores as low as 16 and 8 respectively for the two groups

After diastase digestion for 1 or 2 hours the PAS



Fig 6 PAS positive (rating 4) trinucleated proerythroblast ×1215 (Patient 9)

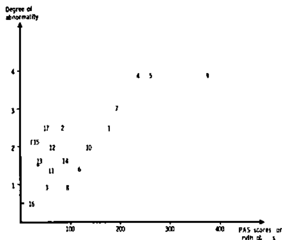


Fig 7 Degree of abnormality in proerythroblasts and basophilic erythroblasts related to PAS scores for the same cells Figures within parentheses indicate case numbers

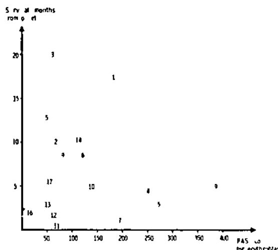


Fig 8 Duration of the disease related to PAS scores for proerythroblasts and basophilic erythroblasts Figures within parentheses indicate case numbers

scores were as listed in Tables III and IV After digestion for 1 hour many PAS positive immature erythroblasts were still seen in patient 9 and a number of more mature PAS positive erythroblasts and a certain PAS positivity in some mature granulocytes persisted in all three cases After digestion for 2 hours this PAS positivity had almost completely disappeared

There was no significant correlation between the PAS score and the survival time from the onset of the disease (Fig 8) However it should be noted that the disease ran a rapid course in the three patients (nos 4 5 and 9) with the most abnormal and most intensely PAS positive erythroblasts while it was subacute in two patients (nos 3 and 15) with low scores Two other patients (nos 11 and 13) with low PAS scores revealed intense proliferation of the granulopoietic tissue

No definite relationship between evidence of haemolysis (28) and PAS positivity was found Among the six patients (nos 2 4 5 6 10 and 16) who had low serum haptoglobin levels three (nos 5 6 and 16) also revealed reticulocytosis and increased serum bilirubin values A strongly positive PAS reaction was found in patients 4 and 5 whereas the erythroblasts in patients 2 6 and 10 were only weakly to moderately PAS positive In patient 16 in whom very intense haemolysis with markedly reduced erythrocyte survival time was present only extremely few weakly PAS positive erythroblasts were seen

In pernicious anaemia folate deficiency and haemolytic anaemias we have found only very few PAS positive erythroblasts Similarly most of the myeloma cells which we have studied so far have shown a negative staining reaction or as far as a

Table III *PAS positivity in young erythroblasts after diastase digestion for one (A) and two hours (B)*

Case No	PAS score for 100 consecutive erythroblasts: Distribution of positivity 0	1	2	3	4	Total score
4	99	1	0	0	0	1
8	98	2	0	0	0	2
9	32	11	30	18	9	161
4	100	0	0	0	0	0
8	100	0	0	0	0	0
9	100	0	0	0	0	0

Table IV *PAS positivity in more mature erythroblasts after diastase digestion for one (A) and two hours (B)*

	Case No	PAS score for 100 consecutive erythroblasts: Distribution of positivity 0	1	2	3	Total score
A	4	49	42	10	1	65
	8	25	68	7	0	82
	9	24	56	12	8	104
B	4	88	12	0	0	12
	8	88	12	0	0	12
	9	92	8	0	0	8

few cells were concerned a weak local PAS positivity A more detailed report of these findings will be given in a separate paper (30)

In order to illustrate the value of checking the correctness of the staining technique by means of cells from the granulopoiesis brief comments on one of our patients will be of interest

Case 5 is identical with a previously reported case of erythroleukaemia (29) In the study of the PAS stained smears at that time we were surprised to see that the highly immature and morphologically distinctly abnormal erythroblasts were PAS negative Even with some tolerance the PAS scores obtained for proerythroblasts plus basophilic erythroblasts and intermediate plus late erythroblasts were at most 0 and 3 respectively since the cytoplasm of three of the more mature erythroblasts apparently had a faint pink tinge After a subsequent evaluation of a series of PAS stained smears repeat microscopy revealed that none of the few more mature cells from the granulopoiesis which were present in the first smear studied were PAS positive i e owing to a technical error in the laboratory it had not been subjected to staining with Schiff's reagent Renewed PAS staining of two stored mounted Giemsa stained smears from this patient revealed intensely PAS positive erythroblasts (Tables I and II)

DISCUSSION

PAS positive erythroblasts were found in all 17 pa[illegible] and the variations in PAS positivity of the [illegible]lasts ranged from 2 to 99% Similarly Kawakita (14) observed PAS positive erythroblasts in 31 of 32 patients with Di Guglielmo s syndrome the percentage of positive cells varying from 2 to 80 while Baldini et al (2) reported a range of variation from 1 5 to 32

In agreement with several other investigators we conclude from these results that the presence of PAS positive erythroblasts lends support to a diagnosis of Di Guglielmo s syndrome (1 2 10 12 13 14 17 18 21 22 24) However a very low score—or even a negative reaction—does not exclude this diagnosis (12 14 23 32) Strongly PAS positive erythroblasts as observed in many of the patients in our series have apart from Di Guglielmo s syndrome been demonstrated only in iron deficiency anaemia and thalassaemia (12 21) i e diseases which are usually not difficult to differentiate from Di Guglielmo s syndrome

As it may be difficult to distinguish myeloblasts and promyelocytes from basophilic erythroblasts in PAS stained smears a certain number of these granulopoietic cells may perhaps have contributed to the PAS scores for basophilic erythroblasts and proerythroblasts obtained This obviously applies in particular to the two patients (nos 11 and 13) who had the largest number of myeloblasts in the bone marrow (27) This possibility was also emphasized by Hayhoe et al (12)

Like Hayhoe (11) and Giralt and Raichs (10) we found a difference in the type of PAS positive material in the most immature and the more mature erythroblasts in the former always with a clear background of unstained cytoplasm

As already mentioned in PAS staining after diastase digestion it can be decided whether a given positive PAS reaction is referable to the presence of glycogen Our results of diastase digestion (Tables III and IV) confirm that the greater part of—or presumably all—the PAS positive material in the erythroblasts is due to glycogen just as in the neutrophils (cf Tables III and IV with Tables I and II) A period of 1 hour, as suggested by Dacie and Lewis (4) does not seem to be sufficient for complete digestion of the glycogen Like Skjoldborg (25) we must therefore recommend a digestion period of at least 2 hours

We noticed a correlation between the cell morphology in the Giemsa stained preparations and the PAS positivity in that the most abnormal erythroblasts were strongly PAS positive Such a correlation has also been observed by other investigators (21) and it has in addition been demonstrated that particularly highly immature erythroblasts often have a very high content of acid phosphatase (3 9 13 15 23)

A correlation although not statistically significant possibly exists between the PAS positivity of the erythroblasts and the course of the disease so that the disease apparently runs a rapid course in patients with the most abnormal and most intensely PAS positive erythroblasts while it is subacute in some patients with low PAS scores Similarly Hayhoe et al (12) and Kawakita (14) noticed a weak reaction involving only a small proportion of erythroblasts in a few cases of subacute or chronic erythraemic myelosis In acute cases the PAS reaction appears to offer a sharper diagnostic criterion (12)

In the same patients who had intensely PAS positive erythroblasts PAS negative erythroblasts were also observed (Tables I and II) Based on similar observations Skinnider and Ghadially (24)

advanced the hypothesis that PAS positive erythroblasts may be derived from an abnormal clone In our opinion there is no definite relation between PAS positivity and the presence of an abnormal clone since our patient 16 who had the lowest PAS score (Tables I and II) had an abnormal clone with 45 chromosomes in his bone marrow whereas another patient (no 3) who had an appreciably higher score did not reveal any abnormal stem line

PAS positive erythroblasts are apparently damaged cells which have been arrested in their development (20) The fact that so many of the erythroblasts from patients with Di Guglielmo s syndrome show intense PAS positivity presumably provides evidence in support of the presence of a profound metabolic derangement of these cells and hence in the production of erythrocytes which fits the picture of the ineffective erythropoiesis demonstrated in this disease (6 20) It seems likely that acquired intrinsic metabolic defects in the erythroblasts are present in Di Guglielmo s syndrome (5) The PAS positivity which must be a manifestation of glycogen accumulation suggests a defect in the glycolytic enzymes (2 16 22) Some enzymatic defect may perhaps also be responsible for a possible inability of the cells to take up or utilize the vitamin B_{12} (2 7 8) or folate which is usually present in sufficient amounts in these patients This might also offer an explanation of the striking similarity which is often seen between the megaloblasts in these vitamin deficiency diseases and the Di Guglielmo cells

In pernicious anaemia and folate deficiency the PAS positivity of the erythroblasts is not nearly so conspicuous (21 30) As however some patients with Di Guglielmo s syndrome have very low PAS scores or even a score of 0 a supposed defect in the glycolytic enzymes in the erythroblasts cannot in all cases be the only abnormality—rather one of many possible defects in these cells

Just as in typical myeloblastic leukaemia (31) some patients with Di Guglielmo s syndrome have revealed a defective Hb synthesis with the occurrence of increased amounts of Hb F and Hb H (19 22) and in vitro experiments in some patients have provided evidence suggestive of an abnormality in the enzymes which are essential in the synthesis of haeme (19 26) and globin (19)

In recapitulation it may be concluded that PAS staining of the erythroblasts is of value in the diagnosis of Di Guglielmo s syndrome as PAS positivity supports the suspicion of the disease and the intensity of the staining reaction may be a pointer to its course

ACKNOWLEDGEMENT

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The Lipoprotein and Lipid Pattern in Xanthomatosis

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ABSTRACT The relation between xanthomatosis and plasma lipid and lipoprotein concentrations has been studied in 336 subjects (172 males and 164 females) with xanthomatosis With a few exceptions these patients were either born or lived in Østfold county, which has a population of about 220 000 Xanthomas had made their first appearance in half the subjects by the age of 48 years The most frequent localizations of xanthomas were the extensor finger and the Achilles tendons, the former site being more common in males and the latter in females Tubero tendinous xanthomas were found in 7% of the cases whereas only one subject had tuberous xanthomas without tendinous involvement Xanthelasmas were found in 1/3 of the females and 1/6 of the males The size of xanthomas and the number of xanthoma sites increased with age and cholesterol levels Cholesterol concentrations were (or had been) increased in 5/6 of the subjects with xanthomathosis Normal cholesterol was combined with increased triglyceride values in 5% of the individuals A further 6% had normal cholesterol and triglyceride values together with raised LDL cholesterol concentrations whereas the remaining subjects had normal lipid levels There was no evidence of homozygosity for the gene(s) for type II hyperlipoproteinaemia The type III hyperlipoproteinaemia pattern was not seen Type II A and II B electrophoretic patterns were seen in 68 and 13% of those tested respectively Changes in lipoprotein patterns with treatment were frequent especially in males and the original frequency of type II A is probably higher

Xanthomatosis usually reflects severe longstanding disorders of lipid metabolism commonly exhibiting high plasma cholesterol levels abnormal lipoprotein pattern and an increased risk of atherosclerotic heart disease It has been known for many years (5 10) that xanthomatosis with hypercholesterolaemia may occur as an autosomal dominant trait

The purpose of the present investigation was to study the plasma lipid and lipoprotein pattern in patients with xanthomatosis from Østfold county in South Eastern Norway

MATERIAL AND METHODS

Demographic data Østfold county covers 4 180 km² with 221 000 inhabitants as of Dec 31 1974 (11) The area is served by 5 general hospitals Of the subjects with xanthomatosis 291 (86 6%) had been born in Østfold and 307 (91 4%) were living there at the time of the investigation 12 (3 6%) did not belong to either of these categories

Ascertainment of probands The probands with xanthomatosis were ascertained as follows (A) From hospital records Records obtained from all of the five hospitals serving the area were searched for patients with a diagnosis of (*a*) xanthomatosis (*b*) hyperlipidaemia and (*c*) coronary heart disease in whom the first symptoms had been seen before the age of 50 years The last two categories were searched for the occurrence of xanthomatosis (B) From other sources General practitioners and specialists in internal medicine ophtalmology pediatrics and dermatology in Østfold were asked to notify the author about such cases under their care

Description of probands Medical records were obtained of altogether 345 patients (Table I) and 265 of these were seen personally 37 of the others had died 11 were unwilling to participate and 7 had moved

All patients showing xanthomas who were alive on Nov 1 1969 were accepted as probands irrespective of the lipid levels or clinical symptoms Subjects with xanthelasmas as the only manifestation were not included Two female patients developed tendon xanthomas under l-thyroxin treatment for hypothyreoidism They were adequately controlled as judged from their clinical condition and from laboratory tests and were included among the probands

All probands were submitted to a clinical examination

[1] Research fellow The Norwegian Council on Cardiovascular Diseases

Table I *Number of subjects studied for xanthomatosis (probands)*

Result of present investigation	Diagnosis before present investigation: Xanthomatosis	Hyperlipidaemia (without xanthomas)	Coronary heart disease before 50 y [a]	Total
Xanthomatosis	146	4	–	150
Xanthomatosis present patient dead	35	–	–	35
Xanthomatosis present? patient dead	2	–	–	2
Xanthomatosis absent	18	95	25	138
Xanthomatosis present probably secondary to other disease	2	–	–	2
Unwilling to participate	6	5	–	11
Moved from or not living in the county (without 1st degree relatives in the area)	7			7
Total	216	104	25	345

[a] Without additional diagnosis of xanthomatosis or hyperlipidaemia

with special attention to xanthomatosis and symptoms and signs from the cardiovascular system As the clinical differential diagnosis between normal and affected tendons was sometimes difficult to make only definitely enlarged tendons were scored as affected

Dead probands In addition 35 decreased persons who had had xanthomas but who had died before the start of this study and did not have first degree relatives registered as probands were treated as a separate group These *dead probands* included 2 subjects who were seen as above but who died after Nov 1 1969 and before blood samples could be obtained

Ascertainment of secondary cases All living first degree relatives of the living and dead probands were con[...] for investigation and were examined in the same [...] as the probands whenever possible Subjects with xanthomatosis in this group are called *secondary cases*

More distantly related subjects were contacted if feasible to obtain as complete information as possible about all cases of xanthomatosis in the area Further details of this search will be given later

Table II *95th percentiles for serum cholesterol and triglyceride values (mg/100 ml) for presumably healthy Norwegians (except when marked) in different age groups*

	Age group 15–19	20–29	30–39	40–49	50–59	60–69
Males						
Cholesterol	265	314	341	383	373	384
Triglycerides	164	252	230[a]	303	303	195[a]
Females						
Cholesterol	271	295	300	360	421	448
Triglycerides	131	131	195	169	169	173[a]

[a] Based on Swedish data from Carlson and Lindstedt (4)

Supplementary information The information obtained from the patients and their relatives was confirmed or supplemented by hospital records contact with their physicians and relatives and by death certificates

Although 48 of the secondary cases and 33 of the dead probands were not seen personally the presence of xanthomatosis seemed to be well established in all patients The clinical records were judged as complete in 51 cases and incomplete but sufficient in 30 of these 81 patients Thirty four (71 %) of the 48 secondary cases not seen were dead and of the remaining 14 11 subjects could not be seen for geographical reasons Three patients were unwilling to cooperate but information was obtained from their physicians

Blood samples All subjects were seen as out patients Blood samples were drawn at least 3 months after a sustained myocardial infarction or other serious disease with the exception of two cases Lipid-lowering drugs or diet were not discontinued prior to sampling but instructions were given to stop low calorie diets temporarily

All blood samples were drawn by venepuncture after more than 12 hours fast Most samples arrived at the laboratory within 4 hours of sampling Serum or plasma was then separated from the corpuscular elements by centrifugation On some occasions serum was pipetted off prior to postage Such samples always arrived within a few days (5 days at the most) Fresh sera or plasma were used for lipoprotein electrophoresis and aliquots were frozen at −28°C until lipid determinations could be performed

Lipid and lipoprotein determinations Total serum cholesterol triglyceride and lipid phosphorus concentrations were determined as described previously (9) Lipoprotein electrophoresis was performed in agarose gel (8) and a visual evaluation was made The lipoprotein fractions were estimated from densitometric recordings The low density lipoprotein (LDL) cholesterol concentration (mg/100 ml) was estimated from the formula LDL cholesterol=Total cholesterol−triglycerides/5-45 (9) A WHO pattern designation (1) was assigned to the electrophoretic strip Preparative ultracentrifugation of

Table III *Age and sex distribution of 336 subjects with xanthomatosis*

	Age group								
	0–19	20–29	30–39	40–49	50–59	60–69	70–79	80–99	Total
Males									
Probands		2	6	13	34	25	–	–	80
Dead probands									23
Secondary cases (living)		8	8	12	15	5	1	1	50
Secondary cases (dead)									19
Total									172
Females									
Probands		2	6	8	20	26	7	1	70
Dead probands									12
Secondary cases (living)	5	10	9	13	10	12	7	1	67
Secondary cases (dead)									15
Total									164

plasma was performed at density 1 006 g/ml at 105 000 *g* for 16 hours

Previous cholesterol and triglyceride determinations As most subjects were under medical care at the time of the present investigation serum cholesterol determinations had been performed previously in the majority of cases but few patients had been examined with respect to triglycerides Serum cholesterol had been determined by the method of Carr and Drekter (3) which has been shown to give results very similar to those obtained with the present method (9) For most patients more than one cholesterol value was available The highest recorded level will be used in the following The results given for deceased subjects are such values Serum lipoprotein electrophoresis had been performed previously in only a very few instances

Normal limits We have previously reported the lipid and lipoprotein values for the general population in the area where the patients live (9) The 95th percentiles of these reference values were used to discriminate between normal and increased levels The term probably increased signifies that the data are insufficient to permit a firm statement in the case at hand The 95th percentiles for serum cholesterol and triglycerides are given in Table II A few of the triglyceride values were calculated from the data published by Carlson and Lindstedt (4) when the collected reference material was insufficient for that age group

Statistical analysis χ^2 tests were used for comparison between distributions when appropriate with Yates correction Comparisons between mean values were made by Student s *t* test allowing for unequal variances Wilcox

Table IV *Age at first appearance of xanthomas*

	No information	No of subjects in age group								
		0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	Total
Males										
Probands		1	2	7	9	24	24	13		80
Dead probands				1	5	8	8	1		23
Secondary cases	5		3	10	11	21	14	4	1	69
Total	5	1	5	18	25	53	46	18	1	172
Females										
Probands			8	4	5	15	25	11	2	70
Dead probands					1	2	5	3	1	12
Secondary cases	7		9	13	9	18	12	10	4	82
Total	7		17	17	15	35	42	24	7	164
Males and females (maximal cholesterol value >500 mg/100 ml)	3	1	7	11	19	34	31	10	2	118
Males and females (maximal cholesterol value <500 mg/100 ml)	9		15	24	21	54	57	32	6	218

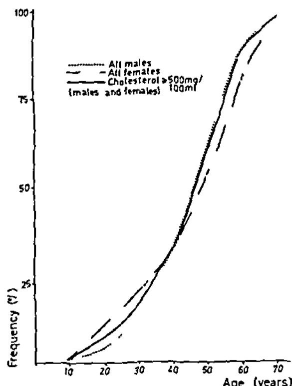


Fig 1 Cumulative frequency of xanthomatosis with age

on s test was used however when the *F* ratio between the variances was significant *P* values below 0 05 were considered statistically significant

RESULTS

and sex of patients with xanthomatosis The age and sex distribution of the 336 subjects with xanthomatosis is given in Table III Approximately equal numers of males and females were found though there were more males among the probands and more females among the secondary cases

First appearance of xanthomatosis The approximate age at which the patient or the physician first noticed xanthomas is given in Table IV together with the time when xanthomas appeared in 118 subjects with previous maximal cholesterol levels exceeding 500 mg/100 ml Among the females early appearance of xanthomas was observed more often in secondary cases than in probands (χ^2=4 01 and 4 88 (p<0 05 for both values) for discovery before 40 or 50 years of age respectively) Other differences within male and female groups (that is between probands dead probands and secondary cases) were not significant Fig 1 shows the cumulative frequency of xanthomatosis with age all males and all females taken together The age of first appearance was very similar in males and females Of the males 50% had their first appearance of xanthomas before the age of 47 years and 50% of the females by the age of 48 5 years However by the age of 60 years xanthomas were more frequent in males than females (χ^2=4 34 1 d f p<0 05) other differences were not significant The plot for occurrence of xanthomatosis in males and females with previous cholesterol levels above 500 mg/100 ml in Fig 1 follows that for males closely and differences between these and the remaining subjects were not significant at 40 50 or 60 years of age In this high cholesterol group xanthomas were present in about 2/3 of the males before 50 years and in 95% by 60 years of age

Localization of xanthomas The most frequent localizations of xanthomas are given in Table V No

Table V *Sites of xanthomas*

	Number of subjects with xanthomas at						
	Finger tendons	Achilles tendons	Knees	Elbows	Feet	Xanthelasmas	Total no of subjects
Males							
Probands	70	68	20	13	10	15	80
Dead probands	17	23	3	2	2	4	23
Secondary cases	58	44	19	10	2	11	69
Total	145	135	42	25	14	30	172
Females							
Probands	51	69	21	11	16	24	70
Dead probands	6	10	3	1	0	6	12
Secondary cases	58	62	15	5	3	24	82
Total	115	141	39	17	19	54	164

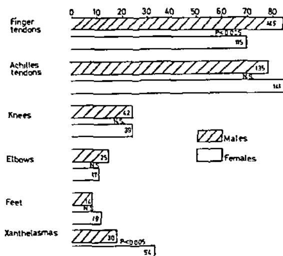


Fig. 2. Localization of xanthomas (%) in 172 males and 164 females.

significant differences were found between the different groups of males or females. Fig. 2 shows the percentages of males and females with xanthomas at different localizations. More males than females had xanthomas at the extensor tendons of the fingers (χ^2=9.64 (1 d.f.) $p<0.005$), whereas Achilles tendon and feet xanthomas were more common in the females, the latter difference being not significant.

The number of localizations of xanthomas was counted in all subjects by defining all extensor finger tendons, both Achilles tendons, etc. as *one* localization each. No significant difference was found in the number of such sites between males and females. Xanthomas were most frequently localized to the Achilles tendon and extensor tendons of the fingers. This combination was found in 66% of males and 61% of females. Xanthomas in finger tendons alone were found in 20% of the males and 12% of the females. Achilles tendon xanthomas were found as the sole localization in 14% of the males and 27% of the females.

Xanthomas located to elbows or knees were always associated with xanthomas in Achilles and finger tendons, although a few subjects stated that their first xanthomas had appeared at the former sites. Much the same number of persons reported their first xanthomas in finger as in Achilles tendons as the initial manifestation of xanthomatosis. Peritendinitis in the Achilles tendons was reported by five subjects (4 females and 1 male).

Unusual localizations of xanthomas were found in three subjects. One subject with severe sequelae of poliomyelitis in the legs had large xanthomas on his nates and shoulders, elbows and knees, as well as tendon xanthomas on fingers and in the planar aponeurosis, but none in his Achilles tendons. In one female, large xanthoma plaques resembling xanthelasmas were seen on the lower part of the neck and the front of the thorax and in the skin folds under the mammae and abdomen. Another female had, apart from tendon xanthomas, lesions in the spatium between her fingers.

Biopsies taken from xanthomatous lesions of tendon and skin in a few patients always had the histological appearance of xanthomas.

Xanthelasmas

Xanthelasmas were significantly more frequent in females (54 cases, 32.9%) than in males (30 cases, 17.4%) (χ^2=9.93, 1 d.f., $p<0.005$). These lesions were most commonly found at the medial aspect of the upper eyelids.

Type of xanthomas

The predominant types were tendinous and subperiostal xanthomas. Tuberous xanthomas were found in 16 males (9.3%) and 9 (5.5%) females (the difference was not statistically significant). All but one of the subjects with tuberous xanthomas also had tendinous xanthomas (tubero-tendinous type). Only one subject had tubero-eruptive xanthomas.

Size of xanthomas

The average size of xanthomas was graded arbitrarily as small, medium or large. In general there was a positive correlation between the

Table VI. *Xanthomatosis in relation to maximal cholesterol values*

Maximal cholesterol value	No. of subjects with xanthomatosis scored as: Small	Medium	Large	Total
Normal	9	43	0	52
High	22	100	13	135
Very high (>500 mg/100 ml)	6	68	59	133
All	37	211	72	370

χ^2=74.81, 4 d.f., $p<0.0005$ for equal distribution of size between the different cholesterol groups.

Table VII *Number of subjects with xanthomatosis in relation to maximal cholesterol value*

	Not tested	Cholesterol concentration (mg/100 ml) 250–299	300–349	350–399	400–449	450–499	500–549	550–599	600–649	650–699	700–749	750–799	800–1 250	Total
Males														
Probands	–	1	4	14	19	12	13	6	8	–	1	1	1	80
Dead probands			1	3	4	3	5	1	2	1	1	1	1	23
Secondary cases	4	4	8	4	17	9	10	3	6	2	–	–	2	69
Total	4	5	13	21	40	24	28	10	16	3	2	2	4	172
Females														
Probands			1	3	13	12	9	9	7	4	3	–	9	70
Dead probands			1	–	4	4	–	–	1	1	–	–	1	12
Secondary cases	4	4	9	7	15	13	10	3	3	5	3	2	3	82
Total	4	4	11	10	32	29	19	12	11	10	6	2	13	164

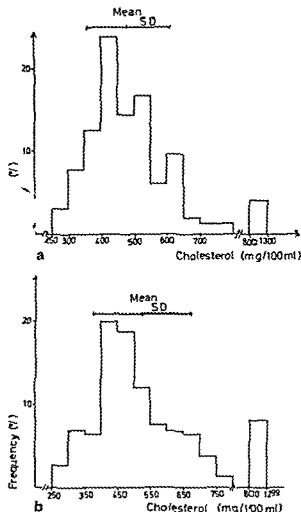


Fig 3 Frequency distribution (%) of maximal cholesterol concentration in 168 males (*a*) and 160 females (*b*) with xanthomatosis

number of xanthomatous sites and the size of xanthomas as well as between the size of xanthomas at different sites in an individual There was a tendency for young subjects to have smaller xanthomas than their elder affected family members There was a highly significant difference in the distribution of xanthoma size between subjects with normal moderately increased or high cholesterol levels (Table VI) Within an age group larger xanthomas were generally found in those with higher cholesterol levels Information about xanthoma size was not available in 10 subjects In another 6 patients cholesterol had not been determined

Lipid and lipoprotein levels

Cholesterol The distribution of the maximal cholesterol values in the different groups of individuals is given in Table VII and Fig 3*a* and *b*

The means and S D of these cholesterol concentrations in different age groups are given in Table VIII Significant differences between the mean values were found between female probands and female secondary cases as well as between all males and all females The mean age was higher in female probands (57 5 years) than in living female secondary cases (50 years) and the mean cholesterol value was lower in females below 40 years than in those aged 40–59 years No significant difference with age was found for males Maximal values above the 95th percentile were found in 272 (81 0%) of the subjects (Table IX) Lipid values were not available for 8 (2 4% of the total series) deceased secondary cases with xanthomatosis whereas a

Table VIII. *Maximal cholesterol value (mg/100 ml) in different age groups of subjects with xanthomatosis (mean ±S.D.)*

	Age group			
	0–39	40–59	60–	Total
Males				
Probands	51[illegible].5±64.1	4[illegible]8.3±11[illegible].3	447.0±99.0	480.1±110.1
Dead probands	5[illegible]5.0	510.7±139.3	550.0±157.5	531.7±134.1
Secondary cases	456.3±96.4	475.0±148.0	470.5±11[illegible].8	474.1±1[illegible]8.7
Total	478.9±97.1	4[illegible]6.5±137.6	469.8±117.4	48[illegible].6±1[illegible]8.7
Females				
Probands	61[illegible].5±159.[illegible]	583.9±[illegible]0[illegible].6	553.7±137.0	5[illegible]0.2±177.0
Dead probands		5[illegible]0.0±158.5	518.9±139.7	503.[illegible]±1[illegible]6.7
Secondary cases	4[illegible].9±98.3	525.0±153.3	508.6±153.3	4[illegible]8.1±163.1
Total	470.3±187.6	553.5±174.3	531.[illegible]±1[illegible]9.0	525.0±147.7

single normal cholesterol value was recorded in 19 (5.7%). At least two normal values (previous maximal level and present) were found in 37 (11.0%) subjects.

An increased cholesterol concentration was found by the *present* lipid determination in 154 of the 263 (58.6%) subjects examined; of the remaining 109 subjects with normal values 6[illegible] ([illegible]3.6%) and 47 (19.7%) had previously increased and normal cholesterol concentrations, respectively.

Triglycerides. The mean, S.D. and range of the triglyceride concentrations in the different groups of patients are given in Table X. As triglyceride concentrations had not been previously determined in most of the deceased subjects, this information was not available in 39 males and 23 females. No significant differences were found between the mean values in the three different categories of males and females, respectively, nor between the values for males and females. The frequency distribution of the maximal triglyceride values is given in Fig. 4a and b.

Supplementary lipid and lipoprotein information. Table XI shows the mean values and S.D. for phospholipids, total lipids, β-, pre-β and α-lipoprotein lipid and LDL cholesterol concentrations of all individuals from whom blood samples were obtained. Levels of β-lipoprotein lipid, LDL cholesterol, phospholipids and total lipids together with α-lipoproteins were higher in females than in males. These differences were all significant. The pre-β-lipoprotein level was higher in males than in females but this difference was not significant. One female with a pre-β lipoprotein lipid level of 1 1[illegible]0 mg/100 ml contributed heavily to the mean and the

Table IX. *Cholesterol values at the previous and present investigation*

Previous investigation (maximal value)	Present investigation. No. of subjects with cholesterol levels			
	Not tested	Normal	Increased	Total
Not tested	8	10	41	59
Normal	9	37	6	52
Moderately increased (<500 mg/100 ml)	23	44	40	107
Strongly increased (≥500 mg/100 ml)	33	18	67	118
Total	73	109	154	336

Table X. *Maximal triglyceride values (mg/100 ml) in patients with xanthomatosis*

	No. of subjects			
	Not tested	Tested	Mean ±S.D.	Range
Males				
Probands	0	80	[illegible]01±94	48–[illegible]00
Dead probands	21	2	125	1[illegible]1–1[illegible]9
Secondary cases	18	51	178±1[illegible]0	3[illegible]–[illegible]60
Total	39	133	191±110	3[illegible]–[illegible]60
Females				
Probands	0	70	178±95	46–650
Dead probands	10	2	175	160–190
Secondary cases	13	69	159±91	30–650
Total	23	141	168±94	30–650

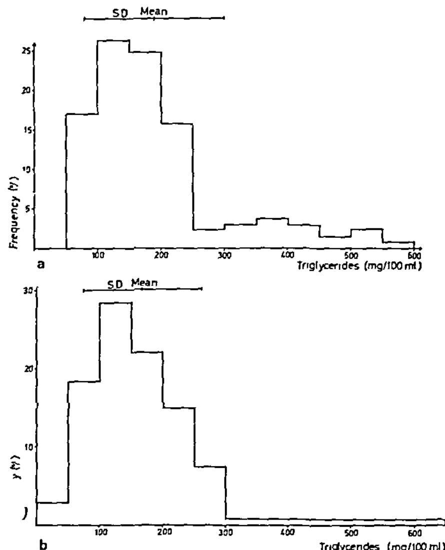


Fig 4 Frequency distribution (%) of maximal triglyceride concentration in 133 males (*a*) and 141 females (*b*) with xanthomatosis

S D When her value was omitted the difference between males and females was highly significant ($p<0.001$) No difference was significant within the various male and female groups for any of these parametres

Lipid and lipoprotein levels in subjects with xanthomatosis but without hypercholesterolaemia

Xanthomatosis was found in 56 subjects who did not exhibit hypercholesterolaemia during or previous to this study Increased triglyceride values were found in 11 of these subjects and another 21 showed increased β lipoprotein lipids and/or LDL cholesterol concentrations No lipid or lipoprotein abnormality was observed in the remaining 24 subjects (8 probands 6 dead probands and 10 secondary cases) for 8 of whom cholesterol values only were available Maximal cholesterol concentrations of 250–299 mg/100 ml were found in 5 300–349 in 7 350–399 in 7 and 400–449 mg/100 ml in 5 of these subjects Lipid lowering treatment had however been instituted in 42 of the 56 patients when the lipid and lipoprotein values were obtained

Diseases which may influence serum lipid and lipoprotein levels

Although glucose tolerance tests were not routinely performed diabetes mellitus had been diagnosed in 8 females (4 probands 2 deceased probands and 2

Table XI *Lipid and lipoprotein concentrations (mg/100 ml) in subjects with xanthomatosis (mean ±S D)*

	No of subjects	Phospholipids	Total lipids	β-lipoprotein lipid	LDL cholesterol	Pre β lipoprotein lipid	α lipoprotein lipid
Males	130	344+8[illegible]	1 110±[illegible]68	659±188	316±111	209+ 93	[illegible]0±45
Females	141	377±76**	1 186±[illegible]81*	811±2[illegible]4 *	37[illegible]±154 *	181±164	[illegible]50±48 *

Difference between mean values significant by Student's t test $*p<0.05$ $*$ $p<0.01$

secondary cases) The diabetic state was of short duration well controlled in all and no subject required insulin Triglyceride values were obtained from 5 of these and were increased in 4 The mean value was 206 mg/100 ml and the S D 88 mg/100 ml The difference between this value and that obtained in other females was not statistically significant

Chronic alcoholism was diagnosed in two brothers one with and one without triglyceride elevation Untreated hypothyreoidism nephrotic syndrome biliary obstruction porphyria or cerebrotendinous xanthomatosis were not detected No case of myelomatosis was found and no M-components were seen in agarose gel electrophoretograms of serum from probands

Effect of lipid lowering treatment on cholesterol levels

At the time of investigation 193 subjects were on a lipid lowering diet (low cholesterol – low fat content with a high proportion of polyunsaturated fatty acids) or were treated with lipid lowering drugs (generally clofibrate or nicotinic acid derivates) In 134 (69%) of these subjects the cholesterol level during the present investigation was lower than the previous maximal value being unchanged in 4 and higher in 23 persons No comparison was possible for the remaining subjects Of the 1[illegible]9 subjects who used a normal diet or received no drugs the cholesterol level was unchanged from the former maximal values in 4 increased in 17 and decreased in 31 subjects while no comparison could be made in the remaining 77 subjects

Lipoprotein electrophoresis

The results of lipoprotein electrophoresis are shown in Table XII The most frequent pattern found was II A (increased amount of β lipoprotein) This pattern was more frequent among females (75%) than among males (61%) Type IIB (increased amount of β as well as pre β lipoprotein)

Table XII *Lipoprotein electrophoretic pattern in subjects with xanthomatosis*

	Number of subjects with electrophoretic pattern					
	Not tested	Normal	II A	IIB	IV	Total
Males						
Probands		[illegible]3*	45	8	4	80
Dead probands	21		[illegible]			[illegible]3
Secondary cases	[illegible]1	7*	3[illegible]	5	4	69
Total	4[illegible]	30	79	13	8	17[illegible]
Females						
Probands		1	5[illegible]	15	[illegible]	70
Dead probands	10		[illegible]			1[illegible]
Secondary cases	13	7	[illegible]	6	4	8[illegible]
Total	[illegible]3	8	106	[illegible]1	6	164*

* Under lipid lowering treatment
* Previously investigated by the author
3 of 7 under lipid lowering treatment
* χ^2 18 49 (3 d f) $p<0.001$ for difference in distribution between males and females

Table XIII *Serum lipid concentrations in relation to lipoprotein electrophoretic pattern in probands*

	No of subjects with electrophoretic pattern				
	II A	II B	IV	Normal	Total
Males					
Cholesterol increased/ triglycerides normal	25				25
Cholesterol increased/ *triglycerides increased*	4	7			11
Cholesterol normal/ triglycerides increased	4	1	4	1	10
Cholesterol normal/ triglycerides normal	12			22	34
Total	45	8	4	23	80
Females					
Cholesterol increased/ triglycerides normal	24				24
Cholesterol increased/ *triglycerides increased*	10	6			16
Cholesterol normal/ triglycerides increased	3	7	2		12
Cholesterol normal/ triglycerides normal	15	2		1	18
Total	52	15	2	1	70

pattern was found in 15 % of the females and in 10 % of the males Type IV (increased amount of pre-β lipoprotein) pattern was seen in 4 7 % of the males *and 3 7 % of the females The difference in the* distribution of lipoprotein patterns between the es and females was highly significant (p<0 001) sex difference persisted after the amalgation of II A and type II B patterns More male than female probands had normal electrophoretic patterns after lipid lowering treatment

A normal lipoprotein pattern was found in 38 subjects 24 probands and 14 secondary cases Of these 3 probands had normal maximal cholesterol and triglyceride values previously The remaining *probands* were treated with diet and/or drugs Fourteen subjects had had elevated cholesterol with normal triglyceride levels or clear plasma whereas 7 had normal cholesterol concentrations without prior triglyceride determination Among the *secondary cases* 8 subjects with normal electrophoretic pattern had previously had elevated cholesterol with normal triglyceride concentrations or clear plasma This was also the case *in two additional* individuals in whom triglycerides had not been determined and the appearance of plasma had not been recorded Normal triglyceride values were found in the 4 remaining subjects

Changing lipoprotein electrophoretic patterns were observed fairly frequently For instance one individual was found to have the following patterns at different bleedings consecutively II A II B IV and normal changes from II B to IV to normal patterns were also observed The frequency of such changes in this material is unknown as complete lipid and lipoprotein data before initiation of treatment were not available in most cases However normalization of elevated cholesterol levels was *frequent An increase in triglyceride concentration* after initiation of a low fat diet was also seen in

Table XIV *Comparison between LDL cholesterol estimates and β-lipoprotein fractions on lipoprotein electrophoresis*

	No of subjects with β lipoprotein electrophoretic fraction			
LDL cholesterol	Normal	Probably increased	Increased	Total
Normal	38	22	4	64
Probably increased	2	14	16	32
Increased	1	6	168	175
Total	41	42	188	271

several patients followed by the change of a previous type II A into a II B pattern

In Table XIII the pattern from lipoprotein electrophoresis is compared with the cholesterol and triglyceride concentrations in the probands. Discrepancies between the electrophoretic pattern expected from the lipid determination and the actual pattern were fairly frequent. Type II A patterns were often seen in samples from subjects with normal cholesterol and triglyceride values. The same pattern was found among subjects with triglyceride concentrations up to 300 mg/100 ml. Type II B patterns were seen with triglyceride concentrations as high as 500 mg/100 ml whereas samples with higher values always gave a type IV pattern.

There was a good correlation between the estimates of LDL cholesterol and the β lipoprotein fraction upon lipoprotein electrophoresis (Table XIV).

No type III pattern was found in the present investigation. Preparative ultracentrifugation was performed on four sera to exclude the possibility that lipoprotein particles with β mobility upon electrophoresis had a density less than 1.006 g/ml.

DISCUSSION

A wide variety of xanthomatosis was observed in the present series. Some subjects had a large number of xanthomas at a young age, others had one or a few small xanthomas late in life. The development of xanthomatosis was in most cases a reflection of elevated lipid/lipoprotein levels.

Walton et al. (12) have recently reviewed the development of xanthomas. According to these authors, hyperlipidaemic plasma leaks into the connective tissue at sites where the *vascular permeability* is increased. This causes uptake of lipoproteins by the tissue histocytes with resulting xanthoma formation. They also found close agreement between the lipid uptake in the arterial, aortic cusp, corneal (arcus formation) and xanthoma tissues.

An interaction of local factors and hyperlipoproteinaemic plasma may explain both the appearance of xanthomas in subjects with normal lipid and lipoprotein concentrations (see below) and the difference in xanthoma distribution between males and females. The higher frequency of xanthomas in the Achilles tendons and on the feet of women may be caused by increased vascular permeability in these areas due to pressure from footwear. The increased frequency of affection of the finger extensor tendons in men may reflect multiple trauma from manual work. The distribution of xanthomas in the male proband with poliomyelitis sequelae also points to the action of local factors. Several subjects had xanthomas localized to scars and this was also the case in a few subjects with normal and slightly elevated lipoprotein concentrations.

As will be reported later, several of the subjects with xanthomatosis and normal lipid concentrations had first degree relatives with xanthomatosis. Small xanthomas limited to a few sites were usually found in subjects without elevated lipoprotein levels. The presence and size of xanthomas were reinvestigated in the majority of such cases and the diagnosis was confirmed in every instance. Discrepancies in scoring of size were very rare.

The frequencies of xanthomas at different localisations probably represent minimal estimates as some subjects were not examined by the author. However, the great majority of such cases were investigated by a small number of very experienced physicians. No difference was found in the pattern of xanthoma localization in probands, who were all seen by the author, in dead probands and secondary cases.

The distribution of xanthomas corresponds closely to that given by De Gennes (6) in his series of 250 subjects. A higher frequency of xanthelasma in women than in men had been reported in other investigations (2, 6).

The age given for the first appearance of xanthomas is probably a maximal estimate as the presence of such lesions was unknown to many subjects. There was little difference between the age for first appearance of xanthomas in subjects with maximal cholesterol values above 500 mg/100 ml and the remaining subjects. This probably reflects the small difference in lipid values for this group compared with the remaining subjects.

Most, but not all, subjects with xanthomatosis showed increased cholesterol values in this or previous investigations. Type II A or II B electrophoresis patterns with elevated cholesterol and normal or slightly elevated triglyceride values were found in the large majority of probands and secondary cases. The remaining patterns were either normal or that of type IV hyperlipoproteinaemia

(4%) The majority of the subjects with normal patterns were treated for lipid disturbances and it was not thought feasible to stop this treatment prior to sampling Normal electrophoresis patterns were found in 23% of males and 11% of females However in about 2/3 of the probands with normal pattern previous lipid values were compatible with a type II A pattern at that time

The frequencies of different electrophoretic patterns prior to treatment are not known as changes in patterns frequently occurred following dietary changes Electrophoresis patterns may also have been influenced by dietary changes or hospitalization (myocardial infarction) prior to collection of the serum sample with the maximal lipid concentration Xanthomatosis was found in 56 (16 7%) of 336 subjects in whom the cholesterol concentration was not known to have been increased Normal cholesterol triglyceride and LDL cholesterol levels were only found in 8 (5 3%) of the probands and 5 of them had cholesterol levels above 350 mg/100 ml Although these values were formally within normal limits the cholesterol (or LDL) concentrations may be higher than optimal from a biological point of view and predispose for xanthoma (and atheroma) formation when local factors interact

Fredrickson and Levy (5) list the following major and minor criteria for heritable type II hyperlipoproteinaemia. Major (A) The pattern of type II hyperlipoproteinaemia and either (B) type II a first degree relative or (C) tendon xanthomas Minor (A) Plasma cholesterol between 300 and 600 mg/100 ml (B) Plasma triglyceride between 50 mg and 500 mg/100 ml (C) LDL cholesterol concentration above 210 mg/100 ml (D) LDL cholesterol and cholesterol concentrations are usually not normalized on diet therapy alone (E) Exclusion of phenocopies

The major criteria for this form of hyperlipoproteinaemia were therefore fulfilled by the large majority of xanthoma cases as type II lipoprotein patterns were (or had been) present in most of them The minor criteria 1 through 3 were also fulfilled in the majority of cases Phenocopies were excluded with the possible exception of the two females who developed tendon xanthomas under adequate treatment of hypothyreosis

The maximal cholesterol values found in the present investigation were higher in both men and women than those reported for type II heterozygotes by Fredrickson and Levy (5) The triglyceride concentration was however raised above the mean level in their patients, a finding that is at least partly explained by the inclusion of patients with type IV pattern in the present investigation The LDL cholesterol levels were higher in females and lower in males than those observed by the same authors The fact that most males of the present series had been treated at the time when the LDL cholesterol concentration was obtained may explain this The lipid concentrations were also higher than those found by De Gennes et al (6)

The frequency of hyperlipidaemia depends on the percentile chosen as the discriminatory point for lipid values There may be good reasons for selecting other percentiles than the 95th as was done in this study In this investigation the effect of using the 90 percentile instead would be that another 2 6% of the subjects with xanthomatosis would be classified as having increased cholesterol or triglyceride values whereas another 5% (or more) of the reference population would be classified as having increased lipid levels

The estimates of LDL cholesterol and β lipoprotein lipid concentrations in this investigation seem to be equally sensitive discriminants in detecting the lipoprotein abnormality More subjects had values outside the reference limits for these parameters than for the plasma cholesterol concentration

The question of homozygosity for a gene for type II hyperlipoproteinaemia arises in 3 subjects because of very high cholesterol concentrations or because of angina pectoris in childhood or because of type of xanthomas Fredrickson and Levy (5) listed clinical criteria for such a diagnosis and these are not fulfilled by any of these subjects One male proband with angina pectoris from the age of 10 had an unaffected mother as well as cholesterol values below 500 mg/100 ml Family data were non informative with respect to homozygosity in the other two patients One female proband had very high cholesterol values (maximal 1 250 mg/100 ml) considerably higher than those of her affected sibs The distribution of xanthomas and age of onset of vascular disease (46 years) were similar to her sibs and not to that expected in a homozygote The third patient a 57 year-old female had a distribution of xanthomas compatible with that described for homozygotes However her clinical state and lipid values were similar to those found in family members who could not be homozygous

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Studies in Asymptomatic Primary Hyperlipidaemia

V *Peripheral Circulation*

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ABSTRACT The occurrence of early signs of *peripheral vascular disease (PVD) of the lower limbs* has been studied with digital pulse plethysmography in 160 men and 123 women above the age of 35, who were all subjectively healthy but were found to have primary hyperlipoproteinaemia (HLP) at a health control centre Quantitative analysis of serum lipoproteins with cholesterol and triglyceride (TG) determination of each of the very low (VLDL), low (LDL) and high (HDL) density lipoprotein classes together with lipoprotein paper electrophoresis was performed Identical investigations were performed on control subjects with non-elevated serum lipids Inclination time (IT) was substantially prolonged in 12% of HLP males and 7% of HLP females The frequency of male subjects with prolonged IT was lower when only LDL was elevated (type II A) than in the other types of HLP The subjects with prolonged IT had three characteristic features 1) Moderate elevations of both serum cholesterol and TG These elevations occurred often in both VLDL and LDL lipoprotein classes IT prolongations were not seen when VLDL or LDL lipoproteins were singly markedly elevated 2) High frequency of smokers 3) Most of the subjects were above the age of 50 The results suggest that the most atherogenic lipoprotein abnormalities to the arteries of the lower limb are on the one hand the relatively TG rich part of the LDL (LDL_1) fraction and on the other hand the relatively cholesterol rich part of the VLDL fraction Furthermore the results stress the deleterious influence *of smoking even in the preclinical stage of PVD*

In recent years attention has been drawn to the serum lipid concentrations or the occurrence of different types of hyperlipoproteinaemia (HLP) in atherosclerotic peripheral vascular disease (PVD) (1 8 11 13) Wheras the importance of elevation of the serum cholesterol concentration in the development of PVD has been stressed in some studies (1 13) other authors have reported high frequencies of elevated serum triglyceride (TG) concentration (8 11) Quantitative estimation of serum lipoprotein (LP) concentrations in PVD has shown increases in very low density LP (VLDL) TG and in low density LP (LDL) TG concentrations but decreases in high density LP (HDL) cholesterol concentrations (8)

Although the frequency of asymptomatic femoral atheroma diagnosed by angiography is reported to be high in subjects with different types of HLP (2) no data are available on the role of elevation of separate quantitatively determined LPs in the development of PVD in the preclinical stage In the present study quantitative LP determinations were performed on apparently healthy subjects with newly detected hyperlipidaemia (15) The occurrence of peripheral atherosclerosis was investigated by volume pulse curve analysis (digital pulse plethysmography) of the lower limbs as this method has proved to be particularly sensitive in the detection of vascular abnormality in early stages of peripheral atherosclerosis (21)

We have called the present approach of detecting preclinical signs of atherosclerotic disease in the presence of a risk factor an interspective study (15) in contrast to prospective and retrospective studies The same approach was also used in a parallel study to establish the prevalence of signs of coronary artery disease This is reported elsewhere (16)

MATERIAL AND METHODS

Subjects and chemical methods

The sampling procedure of the HLP subjects and the methods for serum lipid and LP determination have been

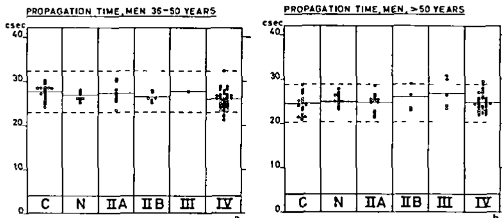


Fig 2 Propagation time in younger (*a*) and older (*b*) male subjects with different types of hyperlipoproteinaemia and in controls (C) ●—smokers O=non smokers The full line indicates the mean value for each group ---= mean ±2 S D of the control group *N*=normal LP type at second blood sampling in spite of hyperlipidaemia at first (screening) sampling

and 24 5±0 4 csec and in females 26 8±0 4 and 24 5±0 3 csec In both males and females mean PT was shorter in the older age groups than in the younger and in general it was shorter in females than in males Mean PT was shorter than that of controls in young males with type IV HLP (25 9±0 3 csec $p<0\ 01$) and in young females with type II B (23 9±0 5 csec $p<0\ 05$) and type IV HLP (24 2±0 4 csec $p<0\ 05$) For the other HLP groups the mean values did not differ significantly from those of the control groups Subnormal PTs were recorded in a few limbs in type IV and N males Among the HLP females subnormal PTs were re corded in altogether 9% In the different groups subnormal PTs were recorded in the following percentages (younger/older) control group 5/0 N 27/5 II A 0/8 II B 50/0 III 0/0 and IV 0/9

Inclination time (IT) (Figs 4 and 5)

The mean values for IT in the younger and older male control groups were 11 1±0 2 and 11 0±0 2 csec The corresponding figures for females were 11 3±0 3 and 11 6±0 2 csec No differences of mean values between different age groups between sexes or between HLP groups and controls were found in either sex

On the other hand 19 (12%) of the male and 8 (7%) of the female HLP subjects had abnormally long ITs (IT prolongation) The IT prolongation in HLP subjects was often pronounced For males IT

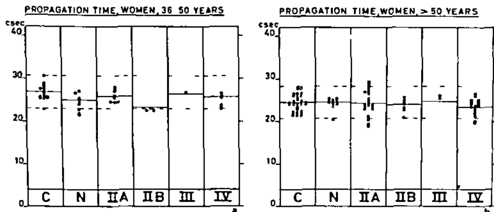


Fig 3 Propagation time in younger (*a*) and older (*b*) female subjects with different types of hyperlipoproteinaemia and in controls (C) Symbols as in Fig 2

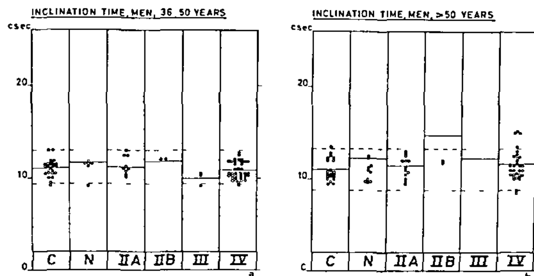


Fig 4 Inclination time in younger (*a*) and older (*b*) male subjects with different types of hyperlipoproteinaemia and in controls (*C*) Symbols as in Fig 2

prolongation was found in the following per centages (younger/older) control group 0/4 N 14/13 II A 0/7 IIB 17/38 III 0/33 and IV 9/18 Thus of the 26 type II A males only one (older group) had IT prolongation The corresponding figures for females were control group 5/0 N 0/0 II A 0/8 IIB 25/13 III 0/33 and IV 0/10

Relative amplitude 25% ($RAMP_{25}$)

igs 6 and 7)

mean values for $RAMP_{25}$ in the younger and ider male control groups were 55±2 and 59±2% respectively The corresponding values in females were 61±2 and 63±1% In men mean $RAMP_2$ tended to be higher in all the older HLP and control groups than in the younger

Mean $RAMP_2$ was higher in males with type IIB HLP than that of controls if younger and older age groups were taken together (66±3% $p<0.05$) No other significant differences between HLP and con trol groups were found

Abnormally high $RAMP_{25}$ values (RAMP eleva tion) were found in 15 (10%) of the male and in 4 (3%) of the female HLP subjects $RAMP_{25}$ elevation

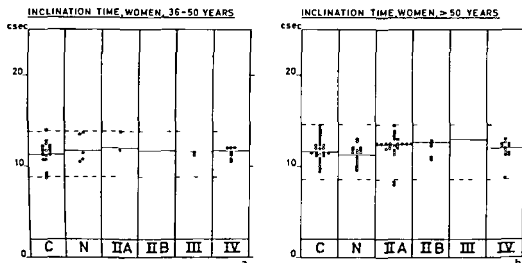


Fig 5 Inclination time in younger (*a*) and older (*b*) female subjects with different types of hyperlipoproteinaemia and in controls (*C*) Symbols as in Fig 2

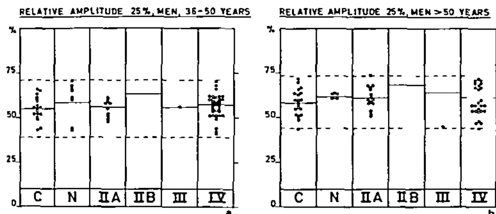


Fig 6. Relative amplitude 25% in younger (*a*) and older (*b*) male subjects with different types of hyperlipoproteinaemia and in controls (*C*). Symbols as in Fig 2.

was often substantial in HLP. In the different types of HLP $RAMP_{25}$ elevation was recorded in the following percentages (younger/older) for males: control group 0/0, N 7/13, II A 0/7, II B 50/25, III 0/33, IV 4/12 and for females: control group 5/0, N 9/0, II A 0/0, II B 25/13, III 0/0 and IV 0/5.

Characteristics of subjects with IT prolongation (Tables II and III)

As IT has proved to be significantly increased even in limbs with only diffuse non-occlusive atherosclerosis and as it is considered to be the most sensitive indicator of early vascular abnormality (21) subjects with IT prolongation will be described in some detail.

IT prolongation was found in 19 (12%) of all HLP males and in 8 (7%) of HLP females. One male and one female control subject also had IT prolongation. Of HLP subjects with IT prolongation 85% were above 50 years.

Prolonged ITs were equally common in the right and left leg. In 52% of the cases IT of both legs was prolonged. Of the males with IT prolongation 84% also had a $RAMP_{25}$ elevation. In 78% the longer $RAMP_{25}$ of the two limbs was found in the same limb as the longer IT. Also in women there was a good agreement between IT and $RAMP_{25}$.

Of the 19 men with prolonged IT 18 were smokers, 16 of them being cigarette smokers. Of the women with IT prolongation 75% were smokers or exsmokers. Of the 3 non smokers 2 had a diabetic k value for the glucose tolerance and one a k value just above borderline. Thus all but one of the sub

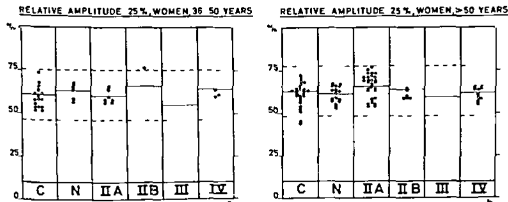


Fig 7. Relative amplitude 25% in younger (*a*) and older (*b*) female subjects with different types of hyperlipoproteinaemia and in controls (*C*). Symbols as in Fig 2.

Table II *Peripheral circulation and clinical findings in subjects with prolonged inclination time*
C=control group N=normal LP type at LP determination in spite of hyperlipidaemia at screening
R=right L=left leg in which the longest IT was recorded

Group	Case no	Sex	Age (y)	IT (csec) Longest		IT (csec) Other	PT (csec) Longest IT	PT (csec) Other	$RAMP_{35}$ (%) Longest IT	$RAMP_{35}$ (%) Other	IVGTT (%/min)	Smoking Cigs / day	Smoking Duration (y)
C	1	♂	63	13 5	L	13 0	25 0	24 8	71	72	–	13	50
	2	♀	43	14 0	R	12 3	30 5	30 7	68	66	–	0	0
N	1	♂	49	13 5	R	10 6	29 3	27 8	83	61	2 11	30	30
	2	♂	50	15 0	R	14 5	25 5	26 0	63	71	1 11	13	25
	3	♂	59	18 5	R	13 8	30 0	25 0	72	64	1 11	10[b]	35
	4	♂	64	21 3	L	20 8	27 8	29 5	90	88	0 38	15	40
II A	5	♂	57	15 0	I	11 8	21 8	23 3	74	52	0 61	0	0
	6	♀	64	16 5	R/L	16 5	29 0	27 0	63	64	2 39	10[c]	40
	7	♀	64	18 0	L	12 5	26 0	25 0	60	73	1 81	3	10
	8	♀	67	15 5	R/L	15 5	26 8	25 8	68	68	1 00	10	40
II B	9	♂	48	14 5	L	12 2	25 1	27 5	71	73	3 05	10	30
	10	♂	57	16 0	L	14 0	27 0	26 5	76	72	1 58	3	15
	11	♂	58	20 5	R	19 0	33 5	31 5	92	93	0 49	20	30
	12	♂	64	25 5	R	17 0	30 0	29 0	72	43	1 48	10[b]	50
	13	♀	48	14 8	R	10 5	27 3	22 5	77	66	1 22	0	0
	14	♀	62	18 0	R	15 5	29 3	26 7	81	71	1 17	8	25
III	15	♂	59	17 8	R	17 0	33 0	30 8	86	81	2 30	20	45
	16	♂	67	14 2	L	14 0	31 0	30 0	76	65	0 80	10	30
	17	♀	64	18 5	L	15 5	29 5	26 0	67	65	0 94	0	0
IV	18	♂	49	15 0	R	12 8	28 5	29 3	82	72	0 96	20	30
	19	♂	50	13 5	L	11 8	30 7	27 8	70	55	1 64	4	30
	20	♂	51	13 5	L	12 3	26 3	25 3	68	55	1 67	20	20
	21	♂	52	14 5	L	13 5	33 0	27 0	79	70	1 03	30	35
	22	♂	54	15 0	R	12 0	26 0	27 0	76	71	1 03	20	35
	23	♂	56	15 0	R	12 3	26 0	23 8	71	57	0 98	4	40
	24	♂	58	16 8	R	14 0	31 5	29 0	89	62	1 11	20	40
	25	♂	63	15 2	R	14 2	23 7	23 7	67	69	1 07	8[b]	40
	26	♀	59	15 5	L	12 5	20 0	23 0	72	65	0 86	13	39
	27	♀	61	18 0	L	10 8	31 0	24 3	88	58	1 88	20	35

Expressed according to the Minnesota code (16)
[b] Pipe smoker 1 g pipe tobacco was considered equal to 1 cigarette (18)
[c] Exsmoker

jects with HLP and prolonged IT had at least two risk factors

Thirty two per cent of the male group and 25% of the female group with IT prolongation had a λ value below 1 0 at the i v glucose tolerance test (IVGTT) Five subjects had a diabetic glucose tolerance

About 60% of the males and 75% of the females with IT prolongation had ST segment depressions during or after exercise typed as 4 1–4 4 according to the Minnesota code (16) In types II A and II B ST segment depressions of this magnitude were found in all subjects with IT prolongation except in one

ESR was above 25 mm/h in 44% of the subjects

Subjects with prolonged IT were found in all HLP groups Although this means that the subjects with IT prolongation had different types of HLP at the time of typing most of them (21 of 27) were characterized by elevation of both serum cholesterol and TG i e mixed hyperlipidaemia at screening (Table III) If we consider the upper normal limits for cholesterol and TG to be 320 mg/100 ml and 2 2 mmol/l (5) only two of the 19 men with IT prolongation had a normal cholesterol value and only one had a normal TG value Interestingly the man with initially normal serum TG had elevated VLDL TG at typing In women three subjects aged above 50 with IT prolongation had initial serum TG below 2 mmol/l and normal VLDL TG

Resting BP (mmHg)	Exercise ECG ST depression	ESR (mm/h)
115/65	4 1	10
170/75	0	8
110/70	0	16
135/80	0	9
120/60	0	21
150/75	0	20
140/85	4 3	7
115/65	4 3	10
145/95	4.5	13
140/65	4 3	22
135/80	4 3	35
135/70	4 2	6
145/85	4 1	63
130/80	4 3	92
170/85	4 3	10
185/95	4 3	12
145/85	4 4	48
135/80	4 4	10
170/95	4 7	28
145/95	4 5	70
135/95	4 3	30
125/75	0	2
105/80	4 4	26
150/85	4 7	50
135/80	0	7
135/70	4 1	41
125/80	4 3	15
140/85	4 1	47
130/90	4.3	38

concentrations All of them had relatively high VLDL cholesterol i e a VLDL cholesterol/TG ratio above 30 (6)

Some further LP abnormalities were seen in the subjects with IT prolongation Thus 8 of 27 had an elevation of the ratio cholesterol/TG in VLDL Nine had raised LDL TG (6) Finally decreased HDL cholesterol concentrations were found in 6 subjects while HDL TG elevations were seen in 11

DISCUSSION

Digital pulse plethysmography has been shown to be a sensitive method discriminating between normal limb arteries and arteries with even mild diffuse non-obstructive atherosclerotic lesions (21) and could therefore be expected to be useful in an interspective study aimed at detection of early signs of atherosclerosis in apparently healthy subjects

Thus in the study of Zetterquist et al (21) IT and $RAMP_{25}$ were significantly increased already when the arteriography showed only slight diffuse atherosclerotic changes but were less sensitive in discriminating between different degrees of more advanced lesions such as stenosis and complete obstruction

PT decreases with age a finding which is held to reflect an increased stiffness of the vascular bed with normal ageing (17 19) PT is also related to heart rate decreasing with increase in heart rate (own observations) In the present study PT was significantly negatively correlated ($p<0\ 001$) independently to both age and resting heart rate In the study of Zetterquist et al (21) PT did not differentiate between normal and diffusely atherosclerotic legs but was increased in the presence of stenotic lesions Complete obstruction was accompanied by a further significant increase Thus PT is influenced by several factors with opposite effects being decreased by increases in age and heart rate and increased by atherosclerotic lesions of increasing severity Therefore it could not be stated with certainty why PT shortening was seen in the younger but not in the older group of type IV men A possible explanation could be that an affect of premature ageing present in the younger group was counteracted by more severe lesions in the older group a contention supported by the fact that several subjects in this group had substantial IT prolongations

Whereas PT was seldom abnormal 10% of the individuals of the HLP groups had IT and $RAMP_{25}$ recordings markedly differing from those of the control groups (Figs 4 and 5) This indicates that the values recorded were signs of vessel wall abnormality and not just extremes of a normal distribution

Although pathological ITs were recorded in a number of different types of HLP some common features were found regarding serum lipid and LP concentrations The majority presented with elevations of both serum cholesterol and TG concentrations i e with mixed hyperlipidaemia

The LP analysis showed that a disordered TG metabolism was common in subjects with IT prolongation not only because of the high frequency of

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Studies on the Serum Lipid and Lipoproteins in Steatosis of the Liver

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ABSTRACT In a medical department, specialized in liver diseases, 45 patients (33 men and 12 women) suffering from steatosis of the liver have been examined with reference to serum lipid abnormalities Twenty-eight of the patients were chronic alcoholics Annual check ups, including biopsy of the liver were made to evaluate the development of the steatosis The patients did not receive treatment with drugs or diet Thirteen patients showed hypercholesterolemia, 16 increased serum triglyceride and 8 increased serum phosphorus lipid Two showed a type I lipoprotein anomaly and 33 a type II like pattern, defined as increased β lipoprotein, but normal serum cholesterol The type II lipoprotein anomaly, defined as increased β lipoprotein associated with increase of serum cholesterol, was found in four cases Three had a type III and three a type IV abnormal pattern with increased pre-β-lipoprotein and serum triglyceride levels Correlation analysis revealed correlations between pre-β lipoprotein and serum triglyceride as well as between serum triglyceride and serum free fatty acid levels

Although the etiology of liver steatosis is unknown or only partly understood this abnormality has been linked with excessive drinking of alcoholic beverages (8) In this respect ethanol seems to induce changes in liver metabolism enhancing Na^{+}–K^{+} adenosine triphosphatase (11) boosting H_2O_2 production and increasing the protein and phosphorus lipid contents of the smooth reticulum of the liver (10) Furthermore ethanol enhances the hepatic glycerophosphate acetyltransferase (13) and induces hypertriglyceridemia and triglyceride (TG) accumulation in the liver (9 18)

In keeping with what may be the case in alcoholism a type IV pre β lipoprotein anomaly (4) is characterized by hypertriglyceridemia Therefore it may be of some interest to elucidate the serum lipoprotein pattern of patients with steatosis of the liver and to correlate this pattern to the TG level especially in cases in whom TG accumulation in liver and serum is due to chronic alcoholism The *present study is devoted to this elucidation*

MATERIAL

The material consists of 45 patients 30 men and 10 women with biopsy verified steatosis of the liver and 3 men and 2 women with previous steatosis who have now developed normal morphology of the liver Table I shows the different degrees of fatty changes of the liver as well as the results of the glucose tolerance test fractionated lipids in serum electrophoretic characterization of serum lipoproteins and the type of hyperlipoprotein anomaly The table also indicates whether the fatty changes of the liver are related to alcoholic abuse Alcoholism was defined as an alcohol intake of more than 50 g daily during the preceding 5 years

METHODS

Assay of serum lipoprotein pattern The serum lipoprotein pattern was separated semiquantitatively and assayed in 1% (w/v) agarose in 0 05 M Na barbiturate (pH=8 0) containing 0 003 M $CaCl_2$ 10 μl fresh serum from individuals fasting for more than 14 hours was applied in a 1 cm trough made in 1 mm thick gel layered on a glass plate (19×10 cm) 2 5 cm from the cathodic edge The electrophoretic separation was made in a water-cooled plastic chamber where water sprayed the lower surface of the glass plate during the run The electrophoresis was made at 10 V/cm for 2 hours Then the pattern was fixed in 50% glacial acetic acid for 5 min and dried below filter paper (Watman No 2) at 22°C The slides were stained in Sudan

Table I *Cl n cal and laboratory data of the pat ents invest gated*

x=Normal serum l poprote n d stribut on in fast ng young nd v duals below 30 years of age y D st nct fraction of chylom crons z Type II l ke pattern def ned as increase in β l poprote n but normal serum cholesterol P=present NP not present n r =normal range p r patholog cal range

Case no	Age (y)	Sex	Weight (kg)	He ght (cm)	Grade of fatty l ver	Glucose toler ance test	Alco hol c abuse	Total choles terol (mg/ 100 ml)	Non-es ter f ed choles terol (mmol/l)	Phospho l p ds (mmol/l)	Fatty ac ds (mmol/l)	Tr glyce r des (mmol/l)
1	38	♀	75 0	166	0	n r	P	322	0 29	5 65	23 7	1 95
2	39	♀	58 4	164	0	n r	NP	284	0 19	3 94	17 0	1 33
3	40	♂	82.5	181	+	n r	P	187	0 24	3 18	17 6	2 13
4	40	♂	82 5	183	++	p r	P	805	0 45	13 6	271 0	59 30
5	42	♀	52 0	152	++	n r	P	44	0 20	3 25	12 7	0 38
6	42	♂	75 0	180	+	n r	P	308	0 33	5 27	35 2	6 14
7	48	♂	77 3	176	+ (+)	n r	P	173	0 22	2 38	9 6	0 41
8	49	♀	72 8	156	++ (+)	n r	P	271	0 25	3 57	17 3	1 26
9	49	♂	94 7	176	++	n r	P	325	0 22	3 66	17 5	1 25
10	50	♂	68 5	171	++	n r	P	351	0 20	3 37	16 9	1 64
11	53	♂	64 2	173	0	n r	NP	287	0 19	2 90	15 3	0 80
12	54	♂	99.5	172	++	n r	P	272	0 18	3 46	14 0	0 85
13	55	♀	45 0	163	++	n r	P	253	0 22	3 98	14 6	0 38
14	55	♂	94 0	182	+	n r	P	223	0 23	2 56	11 1	0 40
15	56	♂	61 1	177	++	n r	P	236	0 18	3 46	15 5	0 58
16	57	♂	89 0	178	++	n r	NP	293	0 24	3 68	19 2	1 72
17	57	♂	75 0	161	++	n r	P	231	0 21	3 61	18 2	1 32
18	57	♂	87 8	178	+ (+)	n r	P	370	0 21	3 58	15 2	0 78
19	58	♂	64 5	172	++	n r	P	207	0 25	3 12	13 1	0 25
20	59	♀	70 6	161	++	n r	NP	229	0 22	3 39	12 9	0 70
21	59	♂	103 0	173	+	n r	P	248	0 28	3 81	31 3	6 20
22	59	♂	83 3	170	+	n r	P	271	0 25	3 65	21 7	2 79
23	59	♂	73 0	189	+ (+)	n r	P	227	0 22	2 80	13 8	0 80
24	59	♂	87 5	169	+ (+)	n r	P	317	0 25	3 90	21 5	1 55
25	60	♂	79 5	177	++	n r	P	312	0 29	5 11	48 4	7 56
26	60	♂	67 0	173	0	n r	NP	237	0 20	2 51	10 6	0 57
27	60	♂	79 1	173	++	n r	P	243	0 30	3 44	17 8	1 55
28	63	♂	85 5	172	++	n r	P	239	0 25	2 95	21 6	2 41
	63	♂	80 5	171	++	p r	NP	247	0 20	2 76	16 1	1 17
	64	♀	61 7	155	++	n r	NP	262	0 17	2 71	16 0	1 18
	64	♂	73 0	168	+	n r	P	246	0 19	2 52	12 8	0 95
3	65	♂	77 7	175	++	n r	P	243	0 4	3 12	13 9	1 10
33	67	♂	71 0	170	++	n r	NP	198	0 23	3 18	14 6	1 58
34	68	♂	99 7	181	+ (+)	n r	NP	297	0 23	3 24	24 4	3 23
35	70	♀	69 0	157	++	n r	NP	256	0 25	2 64	12 8	0 89
36	70	♂	77 0	173	+ (+)	n r	NP	144	0 20	2 97	11 0	0 77
37	70	♂	58 0	158	0	p r	NP	306	0 23	3 42	14 4	0 86
38	73	♂	74 0	155	+ (+)	p r	NP	179	0 23	2 34	12 5	0 75
39	73	♂	67 0	158	+ (+)	n r	P	259	0 21	2 72	16 8	1 1
40	74	♀	67 0	164	+++	n r	P	[illegible]	0 28	3 39	19 7	1 61
41	75	♂	83 2	169	+	n r	NP	223	0 23	3 43	15 5	1 07
42	76	♀	45 1	155	+	n r	NP	248	0 22	3 14	12 6	0 51
43	77	♂	84 0	178	+ (+)	n r	P	221	0 20	2 64	18 5	1 57
44	78	♀	72 9	173	+	n r	NP	247	0 0	2 88	13 9	0 8
45	80	♀	62 5	157	++	n r	NP	300	0 22	3 62	14 8	0 84
Normal values								150– 80 +4	0 0–0 24	2 0–3 5 +0 24	8 0–14 3 +0 37	0 10–1 40 +0 04

Black (2 g/l in 40% aqueous ethanol for 10 m n) and f nally surplus sta n was washed off in runn ng tap water The pattern cons st ng of α pre β and β-l poprote n was semiquantitat vely assayed by photometric scann ng n a V tatron scanning apparatus (5) The normal l poprote n pattern of 44 nd viduals below 30 years of age is presented in Table I (12) The classif cat on of the abnormal l popro te n pattern was made according to the method of Fredrickson and Lees (4)

Furthermore the following assays were made Serum cholesterol (*normal range* 150–280+4 mg/100 ml) non ester f ed cholesterol (normal range 0 20–0 24 mmol/l)

Lipoproteins			
β (%)	pre β (%)	α %	Type of lipo protein anomaly
–	–	–	IV
81 6	8 1	10 2	II z
45 0	38 3	16 6	II z
90 1	–	9 8 v	I
74 1	–	25 9	II.
42 7	50 0	7 2	IV
7? 0	–	28 0	II z
68 6	21 4	10 0	II.
82 7	6 3	10 9	II
–	–	– y	I
72 4	9 2	18 4	II
56 6	22 3	21 0	II.
61 1	25 0	13 9	II z
72 5	7 5	20 0	II.
72 0	10.3	17 6	II z
74 0	15 6	10 4	II
54 4	18 9	26 6	II.
80 7	–	19 2	II
56 2	–	43 8	II.
64 9	10 8	24 2	II.
88 8	–	11 2	III
47 3	40 8	11 8	IV
66 7	21 6	11 6	II z
55 9	25 4	18 6	II z
93 2	–	6 8	III
69 4	12 9	17 7	II.
58 3	26 6	15 0	II z
52 8	–	16 9	II z
6? 5	20 8	16 6	II z
52 7	25 4	21 8	II.
67 1	17 7	15 2	II.
71 2	12 3	16 4	II.
56 4	18 1	25 4	II.
9? 0	–	8 0	III
64 8	19 7	15 4	II.
44 2	18 6	37 2	II.
69 4	–	30 6	II.
70 6	14 7	14 7	II.
63 3	25 0	11 6	II.
77 5	14 6	7 8	II.
57 4	20 4	22.2	II.
79 1	–	20 9	II.
51 4	22 8	25 7	II.
54 7	17 2	28 1	II.
66 2	14 3	19 5	II.
0 x ±9 5	20 6 x ±5 6	18 6 x ±7 2	

(21) serum phosphorus lipids (normal range 2 0–3 5±0 24 mmol/l) (3) serum TG (normal range 0 10–1 40±0 04 mmol/l) (15) serum esterified fatty acids (normal range 8 0–14.3±0 37 mmol/l) (6) and glucose tol rance test (7) Values exceeding the upper range or the means +S D were defined as increased

Chemicals

Unless otherwise stated all chemicals were of the highest obtainable purity from British Drug Houses

Statistical evaluation

The statistical evaluation was made on an Olivetti Prog ramma No 101 using data sheets for calculating coefficients of correlation and for regression analysis The slopes of the regression line were evaluated where they differed significantly from zero Non parametric tests were also used (20)

Histological evaluation

The histological graduation of the fatty changes in the liver was quantified as follows +the biopsy specimens contained fatty vacuoles but on an average in less than one third of the cells ++the specimens contained fatty vacuoles in one third or more but in less than two thirds of the cells +++the specimens contained fatty vacuoles in two thirds or more of the cells

RESULTS

Table I presents the data 13 patients showed an increased serum cholesterol 8 an increased serum phosphorus lipid content and 16 an increased serum TG

Of the patients studied the great majority (n=33) showed a type II like pattern defined as increased β lipoprotein but normal serum cholesterol Four patients had a type II pattern with a predominant β lipoprotein and increased serum cholesterol Only two displayed a type I pattern with a distinct fraction of chylomicrons Three had a type III pattern with the pre β lipoprotein and β lipoprotein emerging together in one more or less common fraction The type IV pattern with a predominant pre β-lipoprotein accounting for more than 30% of the total peak area was found in three cases

Concerning the relationship between types III and IV and the serum TG level all cases with type III and type IV patterns showed increased serum TG levels Increased serum cholesterol was found in two cases of type III and in two of type IV The type II lipoprotein anomaly defined as increased β lipoprotein associated with increased serum cholesterol was found in four cases

A comparison of alcohol consumption with serum TG level indicates that of the 28 patients whose alcohol consumption was verified as abnormally high 13 showed an increased serum TG level Only three of 17 patients without clinically verified alcohol addiction showed hypertriglyceridemia

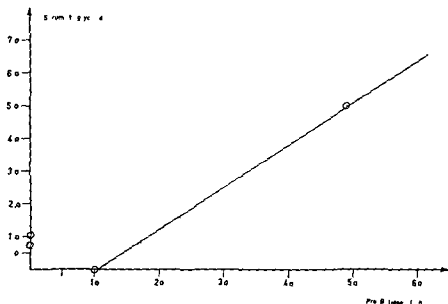


Fig 1 Correlation between serum TG (mmol/l) and pre β lipoprotein (%) (Spearman s correlation coefficient |R|=0 70198 $p \leq 0\ 001$)

This difference between the serum triglyceride levels in the two groups was significant ($p \leq 0\ 02$ Fischer s non parametric test)

Of the patients with anamnestic information of alcohol consumption and hypertriglyceridemia 5 (38%) were overweight (defined as b wt 10% above the theoretical which in kg corresponds to the number of cm by which height exceeds 1 m) Four of the overweight patients belonged to the group with types III and IV lipoprotein anomalies

Correlation and regression analysis revealed a linear relationship between the serum TG levels and *the percentage of the pre β lipoprotein* (correlation for $p \leq 0\ 001$) (Fig 1) and the serum free fatty acids (FFA) (Fig 2) (correlation coefficient $r = 0\ 95$) The slope of this regression line differed significantly *from zero* ($p \leq 0\ 001$) *The slope of the* regression line showing the serum FFA level as a function of the percentage of the pre β lipoprotein (Fig 3) was however also significantly different from zero ($p \leq 0\ 001$) but the correlation coefficient was only 0 69 A correlation study of the serum level of phosphorus lipids towards the total FFA level also revealed a significant linear correlation with however a slope not significantly different *from zero* (*Fig 4*)

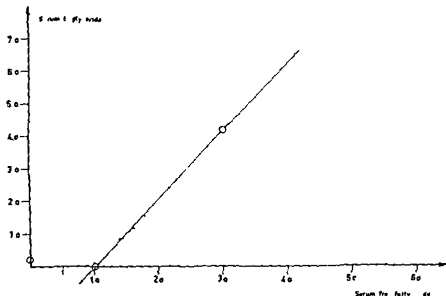


Fig 2 Correlation between serum TG (mmol/l) and serum FFA (mmol/l)

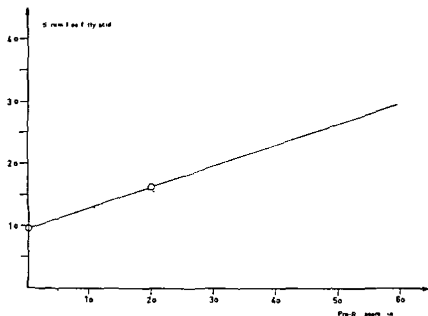


Fig 3 Correlat on betweer serum FFA (mmol/l) and pre β lipoprotein (%) (Spearman s correlation coeff cient |R| 0 48740 p≤0 001)

DISCUSSION

Although 28% of the chronic alcoholics with steatosis of the liver showed hypertriglyceridemia this disorder was not always associated with a type III or a type IV lipoprotein abnormality since only six of these patients had these abnormalities On the other hand the finding of a significant correlation between the serum TG and the serum FFA levels in alcoholics may point to a direct effect of ethanol on the liver and fat tissue synthesis of fatty acids which as precursors of TG may influence the formation of these lipids (2 19) However the no more than moderate correlation between serum TG and the percentage of the pre β lipoprotein fraction on *the one hand but a correlation between serum FFA* and serum TG on the other indicates that the metabolism of apo-pre β lipoprotein may be independent of the fatty acid and TG metabolism Furthermore it may also support the view that the type III and type IV lipoprotein abnormalities are

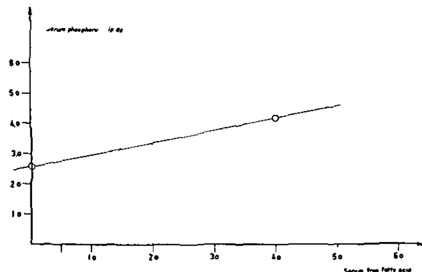


Fig 4 Correlat on between serum phosphorus l pid (mmol/l) and serum FFA (mmol/l) (Spearman s cor relat on coeff c ent |R| *0 093938 p≥0 10)*

basic abnormalities in apo lipoprotein metabolism and not primarily related to abnormalities in TG metabolism anyway not of the type encountered in chronic alcoholics

Only a few cases of the type II lipoprotein anomaly (defined as increased β lipoprotein and serum cholesterol but normal serum TG) were found among patients with steatosis of the liver This may support the idea that steatosis has nothing to do with this lipoprotein anomaly This fact should be stressed since elevated output of β lipoprotein from the liver was found in patients with ethanol induced liver damage treated with androgenic anabolic steroids (16)

The correlation between serum levels of phosphorus lipids and TG and FFA may be explained by an alcohol dependent general stimulation of fatty acid synthesis as well as a stimulation of the acyl glycero and acyl lysophosphatidyl choline transferase (13 17) in liver and fat tissue However recent studies on the redox state of liver cytosole and mitochondria (14) seem to indicate an extrahepatic organ of the triglyceride accumulation in the liver possibly due to a higher influence of ethanol on the acyltransferases of the fat tissue than on these enzymes present in the liver tissue

In conclusion the present data on the lipid abnormalities in hepatic steatosis have shown that in cases where this hepatic abnormality is related alcoholism a correlation occurs between high FFA serum TG and to some extent to serum β lipoprotein levels On the other hand although alcohol is known to stimulate TG synthesis alcoholism associated with steatosis hepatis is not associated with Fredrickson lipoprotein type IV abnormality

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Plasma Aldosterone in Anephric and Non-nephrectomized Dialysis Patients in Relation to Changes in Plasma Potassium without Change in Total Potassium Balance

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ABSTRACT Plasma aldosterone concentration has been measured in 13 patients on regular hemodialysis 7 anephric and 6 non nephrectomized, following infusion of insulin–glucose In all patients the plasma potassium fell about 16% within the first 60 min after the infusion, increasing again to about preinfusion levels within 180 min All anephric patients showed a very low preinfusion value for plasma aldosterone (mean 33 3 pg/ml plasma), which increased significantly ($p<0.01$) after the infusion of insulin–glucose to about 180% of the initial values simultaneously with the fall in plasma potassium concentration A significant inverse correlation ($p<0.01$) was found between changes in plasma aldosterone and potassium The preinfusion plasma aldosterone values were significantly higher ($p<0.01$) in the non nephrectomized than in the anephric group—but of a wide range (26–700 pg/ml plasma) In this group the mean plasma aldosterone concentration showed a significant fall simultaneously with the fall in plasma potassium concentration, but with great variations between patients There was a significant positive correlation ($p<0.005$) between the changes in plasma aldosterone and potassium These studies indicate an extremely sensitive regulation of plasma aldosterone secretion in anephric patients, in all probability positively correlated to changes in intracellular adrenal potassium concentration

There are indications (3 5 12) that in the absence of renin–angiotensin system potassium is the predominant factor in the stimulation of aldosterone production

The following experiments were performed in order to investigate whether the effect of potassium on the aldosterone production was due to changes in the extracellular potassium concentration or to changes in the intracellular concentration Thirteen patients on chronic intermittent hemodialysis 7 anephric and 6 non nephrectomized were given insulin and glucose i v 3 hours before the start of hemodialysis and serial determinations were performed of plasma aldosterone plasma potassium sodium cortisol plasma glucose and BP

Administration of insulin–glucose produces a fall in plasma potassium and at the same time an increase in intracellular potassium concentration This is shown to take place in the muscles (1) and in the liver (7 8) but no investigations have demonstrated this effect on the adrenals It is known however that the intracellular potassium concentration in the zona glomerulosa of the adrenals increases more than that of muscles and liver following potassium loading (2) On the hypothesis that potassium concentration after i v administration of insulin–glucose increases intracellularly in all tissues and thereby also in the zona glomerulosa we considered this as a model to investigate the effect of changes in the potassium concentration of zona glomerulosa on the aldosterone production without changes in total potassium balance in anephric patients

MATERIAL

The investigations were carried out in 13 patients with end-stage kidney disease on regular hemodialysis Seven patients were bilaterally nephrectomized and 6 non nephrectomized

The anephric group comprised 5 women and 2 men with an average age of 39 years (range 31–43) The average time on hemodialysis was 32 months (range 2–55) The renal failure was caused by chronic glomerulonephritis in 4 polycystic kidney disease in 2 and malignant hypertension in one of the 7 patients

Table I *Laboratory data of the anephric and non nephrectomized patients*

	Plasma aldosterone (pg/ml)								Plasma potassium (pg/ml)							
	Preinfusion						Pre dial	Post	Preinfusion						Pre dial	Post
Min	0	30	60	90	120	150	180	dial	0	30	60	90	120	150	180	dial
Anephric patients																
Mean	33 3	60 6	51 7	52 8	45 5	34 1	40 8	17 8	5 4	4 6	4 5	4 7	4 8	4 9	5 0	3 7
±S E M	2 9	15 8	11 2	13 8	10 3	6 4	7 9	1 4	0 21	0 20	0 20	0 18	0 19	0 27	0 23	0 23
Percentage variation	100	181 9	155 2	158 5	136 6	10? 4	122 5	53 4	100	85 1	83 3	87 0	88 9	90 7	92 6	68 5
Non nephrectomi ed patients																
Mean	207 5	98 3	80 6	109 6	115 2	146 4	219 6	65 0	5 1	4 4	4 3	4 5	5 0	5 1	5 3	3 4
±S E M	107 7	40 3	34 6	42 5	47 1	76 3	126 2	25 5	0 29	0 26	0 24	0 22	0 27	0 31	0 27	0 26
Percentage variation	100	47 3	38 8	52 8	55 5	70 6	105 8	31 3	100	86 2	84 3	88 2	98 0	100	103 9	66 7

The non nephrectomized group comprised one woman and five men The average age was 35 years (range 23–55) The average time on chronic intermittent hemodialysis was 23 months (range 1–45) Five patients had chronic glomerulonephritis and one patient had obstructive nephropathy

Dialysis procedure

All patients were dialysed with disposable parallel plate dialyzers (Gambro Lundia or Boe Dawids) during an average period of 10 hours (range 9–11) twice a week They received a diet containing on an average 0 8 g protein/kg b wt 50 mEq sodium 50 mEq potassium and about 800 fluid daily The patients were heparinized during ysis by single heparin injections The total dose per ysis ranging from 15 000 to 25 000 IU The average ght loss during dialysis was about 1 900 g in both roups All patients were in good condition Apart from the fact that 6 patients were HAA antigen positive there was no evidence of hepatic dysfunction

METHODS

All patients were in the supine position throughout the studies Venous blood samples were drawn after 30 min in the supine position 5 min before i v injection of 16 IU crystalline zink insulin and 100 ml 50% glucose administered over a period of 5 min Every 30 min after the injection blood samples were collected for the following 180 min after which the hemodialysis was started Furthermore one sample was collected at the end of the dialysis on an average 800 min after the injection of insulin-glucose BP was measured at every collection of blood samples

Plasma aldosterone potassium sodium and glucose were determined on all samples and in 6 patients plasma cortisol

All patients developed mild symptoms of hypoglycemia 90–120 min after the administration of insulin–glucose At this time all patients were given a further 50 g glucose i v

Plasma aldosterone was determined by a radioimmunoassay described elsewhere (13) The method uses

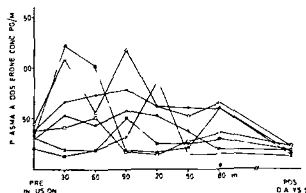


Fig 1 Plasma aldosterone concentrations in 7 anephric patients after infusion of insulin–glucose The arrow at 180 min indicates start of hemodialysis

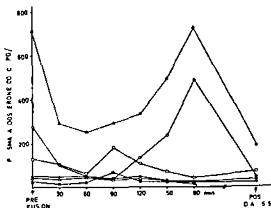


Fig 2 Plasma aldosterone concentrations in 6 non nephrectomized patients on regular hemodialysis after infusion of insulin-glucose The arrow at 180 min indicates start of hemodialysis

Plasma cortisol (pg/ml)							
Preinfusion						Pre dial	Post
0	30	60	90	120	150	180	dial
11 6	9 4	10 4	9 2	13 9	14 8	13 7	6 0
2 17	2 74	1 87	2 52	1 99	2 47	2 03	0 60
100	81 0	89 6	79.3	119 8	127.5	118 0	51 7
13 0	9 3	6 6	9 2	17 8	19 0	14 6	4 8
2 05	0 98	0 19	1 40	0 29	2 60	0 19	2 60
100	71 5	50 7	70 7	136 9	146 1	112.3	36 9

paper chromatography for separation of the steroids Blank value was 1 3±2 8 and sensitivity 6 pg/ml The interassay variability was 13% and the intraassay variability 9 4% An accuracy study on a fixed amount of unlabelled aldosterone added to pool plasma gave a linear correlation (slope=0 9973) The aldosterone antiserum was a gift from The National Institute of Arthritis and Metabolic Diseases Bethesda

Plasma cortisol was measured by a double isotope technique

RESULTS

Fig 1 shows plasma aldosterone concentrations in 7 anephric patients after administration of insulin–glucose In all patients the concentration increased by about 180% of the preinfusion value within the first 30–120 min after the infusion and decreased again to a level close to the intial values within 180 min The mean concentrations and the percentage variations in plasma aldosterone potassium and cortisol are shown in Table I

In the non nephrectomized patients the mean preinfusion plasma aldosterone values were significantly higher than in the anephric group and the range was much larger (Fig 2) Within the first 30–120 min the mean plasma aldosterone concentration decreased in this group as shown in Fig 3 After 180 min the concentrations had returned to about the initial levels (Table I) Three patients with the highest preinfusion values showed a significant fall in plasma aldosterone concentrations two showed no significant change and the patient with the lowest preinfusion value showed an increase in plasma aldosterone concentration as seen in the anephric group

The plasma potassium concentration decreased to about 83 5% of the initial value within the first 60 min after the infusion of insulin–glucose and then increased slowly to about preinfusion values There was no significant difference between the two groups of patients

Fig 3 shows the relationship between the mean plasma concentrations of potassium and aldosterone in the two patients groups As shown in Fig 4 there was a significant reverse correlation ($p<0\ 01$) between changes in plasma concentrations of potassium and aldosterone in anephric patients ($r=-0\ 8228$) while the reverse was found between plasma potassium and plasma aldosterone concentrations in non nephrectomized patients who showed a significant positive correlation ($p<0\ 005$) between potassium ($r=0\ 8587$) and plasma aldosterone

In all patients in both groups plasma potassium fell to about 67% of the initial value after the dialysis due to total potassium depletion from the body The mean plasma aldosterone concentration also decreased significantly in both groups—anephric patients to 53 4% and non nephrec

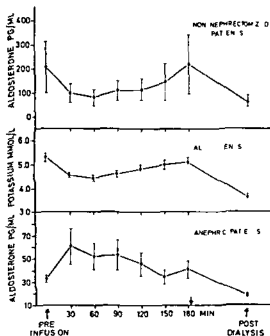


Fig 3 Mean values of plasma aldosterone and plasma potassium concentrations in anephric and non nephrectomized patients after infusion of insulin–glucose The arrow at 180 min indicates start of hemodialysis

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Percentage variation	100	181 9	155 2	158 5	136 6	102 4	122 5	53 4	100	85 1	83 3	87 0	88 9	90 7	92 6	68.5
Non nephrectomized patients																
Mean	207.5	98 3	80 6	109 6	115 2	146 4	219 6	65 0	5 1	4 4	4 3	4 5	5 0	5 1	5 3	3 4
±S E M	107 7	40 3	34 6	42 5	47 1	76 3	126 2	25 5	0 29	0 26	0 24	0 22	0 27	0 31	0 27	0 26
Percentage variation	100	47 3	38 8	52 8	55 5	70 6	105 8	31 3	100	86 2	84 3	88 2	98 0	100	103 9	66 7

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Plasma aldosterone was determined by a radioimmunoassay *described elsewhere (13) The method* uses

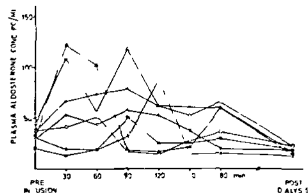


Fig 1 Plasma aldosterone concentrations in 7 anephric patients *after infusion of insulin–glucose The arrow at 180* min indicates start of hemodialysis

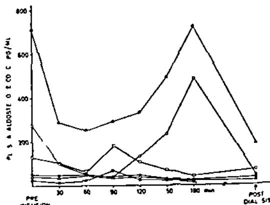


Fig 2 Plasma aldosterone concentrations in 6 non nephrectomized patients on regular hemodialysis after in*fusion of insulin–glucose* The arrow at 180 min indicates start of hemodialysis

Plasma cortisol concentration was measured as an indicator of plasma ACTH variations A reduction was seen for the first 90 min and may reflect the diurnal variation with a fall after the early morning peak (9) Thus variations in the plasma ACTH concentration cannot explain the observed variations in the aldosterone concentration

Plasma sodium was probably not involved as the sodium concentration did not change during the investigations Furthermore the infused volume (100 ml 50% glucose) constitutes less than 1% of the extracellular volume and even when the increase in osmolarity is taken into account the maximal extracellular volume increase will be about 5%

In the non nephrectomized patients the results showed a less uniform pattern The mean preinfusion plasma aldosterone concentration was high with a wide range

In the 3 patients with the highest preinfusion values the plasma aldosterone concentration showed a significant fall while 2 patients showed no significant change and one patient (with the lowest preinfusion value as in the anephric group) showed an *increase in the plasma aldosterone concentration* after infusion of insulin–glucose Still the mean plasma aldosterone concentration showed a significant fall (61 2%) after the infusion although the plasma potassium concentration dropped by 15 8% just as in the anephric group One may suggest that the fall in aldosterone concentration in the 3 patients with high preinfusion values and probably high renin–angiotensin values is due to changes in the plasma renin activity as a direct influence of the slightly increased plasma volume on the renin concentration (6) and/or to changes in the shift of the potassium concentration in the kidneys (10 11) In this case the remaining three patients may have been nearly or completely functionally anephric and thus not able to react to the stimulation In this group with a significant positive correlation between plasma potassium and plasma aldosterone there was also a positive significant correlation when the postdialysis values were taken into account in contrast to the anephric patients in whom the negative significant correlation was changed to no correlation after allowance for the postdialysis values (total body depletion of potassium)

A similar study has been performed on anephric patients by Cooke et al (4) Their results conflict with the present findings despite only minor differences in the procedures They found a fall in the plasma aldosterone concentration after infusion of insulin–glucose in 7 anephric patients with a positive significant correlation between potassium and aldosterone concentrations instead of our inverse significant correlation Their results are quite similar to those in our non nephrectomized group and there seems to be no obvious explanation for the discrepancy

Our results indicate that the regulation of the aldosterone secretion in anephric patients is extremely sensitive and in all probability positively correlated to changes in the intracellular potassium concentration

ACKNOWLEDGEMENTS

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The Effect of Posture on the First Derivative Thoracic Impedance Cardiogram in Patients with Myocardial Infarction

Preliminary Report

Senefro Gabriel and Lars Orö

From the Coronary Care Unit Department of Medicine Karolinska Sjukhuset Stockholm Sweden

In a recent study (1) we measured the cardiac output with the Minnesota impedance cardiograph as described by Kubicek et al (3) in 10 patients with acute myocardial infarction 2–3 weeks after admission to the Coronary Care Unit (CCU) The study included the measurement of cardiac output at rest in the following positions 1) supine 2) 5 min after tilting 30° with the head upwards (30° ↑) 3) 5 min after tilting 10° with the head downwards (10° ↓) 4) supine after i v injection of propranolol The study allowed us to make some interesting observations on the effect of posture on the first derivative thoracic impedance cardiogram wave form (dZ/dt)

Impedance records from each of 4 patients are shown in Figs 1–4 Each record illustrates 1) ECG 2) dZ/dt 3) PCG 4) ΔZ (impedance change during the cardiac cycle) recorded in supine position after tilting 30° ↑ and after tilting 10° ↓

DISCUSSION

The presenting records illustrate some of the abnormalities in the impedance wave form in patients with a stable myocardial infarction The O wave of dZ/dt wave form was found to occur during the phase of rapid filling of the ventricles (4) It has been suggested that a prominent and elevated O wave is associated with a weak ventricle muscle and abnormal filling of the ventricles (2) The prominent O wave in patients 1 and 2 in the supine and tilting 10 ↓ positions indicates abnormal filling of the ventricles This abnormality disappeared on tilting 30°↑ and seemed to be independent of the change in HR probably due to the decrease of the volume load on the heart Similarly the prominent A waves in the supine and tilting 10°↓ positions in patients 3 and 4 may indicate a high atrial pressure (2) They were less prominent in the tilting 30° ↑ position The above mentioned findings may explain why cardiac patients have a lower exercise tolerance in supine than in sitting position

Contrary to the A and O waves notching on the dZ/dt in patients 3 and 4 appeared when the patients were tilted 30 ↑ In both patients it was associated with an increase in HR The diagnostic significance of this abnormality is not clear but may be explained by the asynchronous contraction of the ventricles (2)

The changes observed in the impedance wave form in supine position after i v injection of propranolol were somewhat similar to those observed in the supine position

The presented records illustrate the value of impedance cardiograms in monitoring signs of myocardial weakness The examination of the patient in different body positions may have the advantage of revealing latent abnormality The findings suggest further study to assess the clinical correlates of such abnormalities a study which is under investigation at present

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Patient 1 A 60-year-old man admitted to the CCU on April 26 1974 ECG showed ST elevation suggestive of posterior wall infarction intraventricular conduction delay and cardiosclerotic changes He had signs of heart failure GOT/GPT 37/17 X-ray heart slightly enlarged

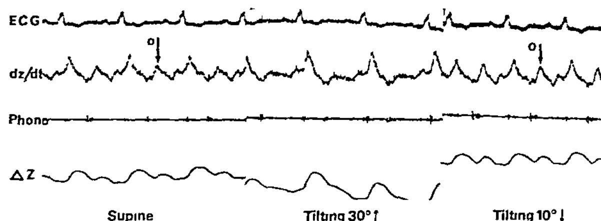


Fig 1 In supine position HR 76 beats/min appearance of abnormal O wave (O↓) the dZ/dt wave form is of low amplitude On tilting 30 ↑ HR 71 beats/min O wave has normal appearance the peak dZ/dt is higher On tilting 10°↓ HR 74 beats/min reappearance of abnormal O wave peak dZ/dt becomes lower

Patient 2 A 59 year-old man admitted to the CCU on June 19 1974 ECG showed posterior wall infarction He had signs of heart failure GOT/GPT 223/50 Exercise tolerance test on July 5 no chest pains no arrhythmia ST elevation corresponding to the infarcted area few ventricular ectopic beats X ray heart slightly enlarged small ventricular aneurysm at the apex

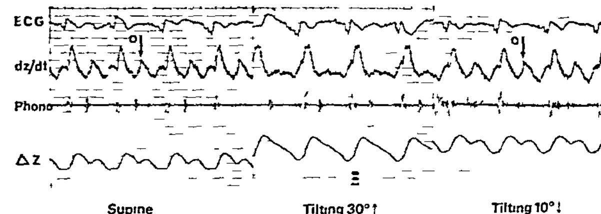


Fig 2 In supine position HR 92 beats/min appearance of abnormal O wave (O↓) On tilting 30°↑ HR 90 beats/min O wave becomes normal On tilting 10°↓ HR 90 beats/min abnormal O wave reappears

Patient 3 A 43 year-old man admitted to the CCU on May 11 1974 ECG showed anterior wall infarction No clinical signs of heart failure the heart was slightly enlarged GOT/GPT 50/21 Exercise tolerance test on May 25 no chest pains no arrhythmia no abnormal ECG changes

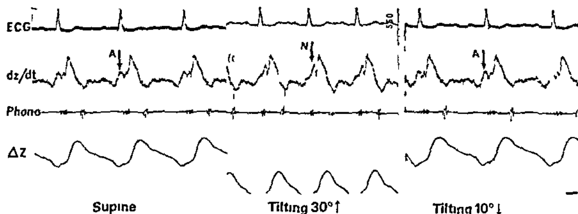


Fig 3 In supine position HR 69 beats/min dZ/dt wave form shows prominent elevated A wave (*A*↓) On tilting 30 ↑ HR 87 beats/min the elevated A wave is lower appearance of notching (*N*↓) on the upstroke of dZ/dt On tilting 10 ↓ HR 63 beats/min reappearance of elevated A wave The notch on dZ/dt has disappeared

Patient 4 A 53 year-old man admitted to the CCU on May 31 1974 ECG showed T changes suggestive of subendocardial infarction Frequent ventricular ectopic beats No clinical signs of heart failure GOT/GPT 24/10 Exercise tolerance test on June 20 no chest pains no arrhythmia no abnormal ECG changes

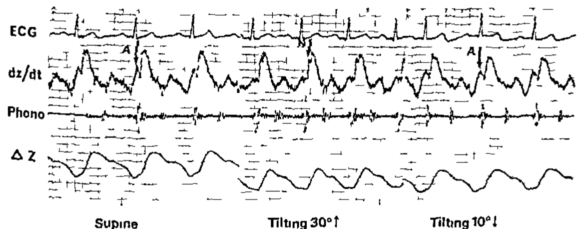


Fig 4 In supine position HR 83 beats/min dZ/dt wave form shows elevated A wave (*A*↓) *On tilting 30* ↑ *HR 94* beats/min A wave normal appearance of notching (*N*↓) on the dZ/dt wave form *On tilting 10°* ↓ HR 84 beats/min *reappearance of elevated A wave less marked notching of* dZ/dt

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Antazoline-induced Immune Hemolytic Anemia, Hemoglobinuria, and Acute Renal Failure

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ABSTRACT **A case of repeated antazoline-induced immune hemolytic anemia thrombocytopenia, hemoglobinuria and acute renal failure is reported The first episode of renal failure occurred after an i v pyelography causing an anaphylactic shock, and the two later episodes were preceded by allergic reaction to drugs Antazoline was given among other remedies but this drug was the only one used for treatment on every occasion The clinical picture and the immunological tests, including an antazoline-dependent Coombs' test, indicate that the blood disorders might have been caused by a type 2 allergic reaction and the renal lesion by a type 3 reaction at least on the second and third occasions**

Acute renal failure does sometimes occur in combination with acute hemolysis but very little is known about the mechanism of different types of nephropathy appearing with hemolysis and hemoglobulinuria The main interest has usually focused upon the hematologic disorder and little attention has been paid to the renal lesion The following presentation deals with a case of drug induced acute hemolysis and renal failure and the aim has been to describe and discuss the possible mechanisms responsible for the erythrocyte destruction as well as for the renal lesion

CASE REPORT

A 58-year-old female clerk had suffered periodically in recent years from mild manifestations of allergic rhinitis mainly due to birch pollen These symptoms had been easily controlled with antihistamines including antazoline

In Nov 1973 the patient had an attack of left sided flank pain suspected as renal colic On Nov 9 she therefore underwent intravenous pyelography (IVP) using diatrizoate meglumine (Urografin®) which immediately led to a Quincke edema and a BP fall to 60 mmHg systolically The symptoms subsided on treatment with 100 mg hydrocortisone and 2 ml antazoline (Antasten®) i v and she was sent home The IVP was normal

On the following night she noticed dark urine and on the next day high fever (41 C) and she was examined at an outpatient clinic The urine was bloody no microscopy was done Ampicillin was given but a urine culture sent simultaneously was later found to be negative Although the fever and the discoloration of the urine disappeared in one day she did not feel well she had nausea passed loose stools and small urine volumes On Nov 22 she was found to have a serum creatinine concentration of 22 mg/100 ml Hb 12 5 g/100 ml ESR 35 mm bilirubin 0 6 ml/100 ml Following admission on Nov 26 the daily urinary output had risen to 2 1 l and remained at that level Urinary findings Addis count 20 mill erythrocytes and 7 mill leucocytes and a proteinuria of 0 7 g/l Hb decreased to 10 2 g/100 ml WBC 11 300 mm³ on admission normalized in a few days but the differential leucocyte count disclosed a rising eosinophilia to a maximum of 12% Platelet count was normal and ESR reached a level of 80 mm

The only drug given after admission was 0 1 g pentobarbital (Mebumal®) every night The patient was recovering continuously and on Dec 8 the serum creatinine had reached a level of 3 mg/100 ml She went home over a weekend and developed urticaria and a slight fever On Dec 10 she had a new bout of red urine which disappeared on her first day back in hospital A cystoscopy on the same day showed blood-stained urine puffing out from both ureters BP was normal Pentobarbital was suspected to have caused the urticaria, but it was not clear whether she had taken some other drug on her home leave On Dec 12 the urinary sediment contained only scattered red cells and a slight proteinuria disappeared within one week Serum creatinine increased to a maximum of 6 2 mg/100 ml but she was not oliguric Hb decreased to 7 0 g/100 ml She had a transient leucocytosis and thrombocytopenia with a lowest count of 76 000/mm³ She had no reticulocytosis Tests of liver function stayed normal Haptoglobin 1 4–0 4 g/l Coombs direct test weakly positive Coombs indirect test negative (3 days after the acute episode) Total com

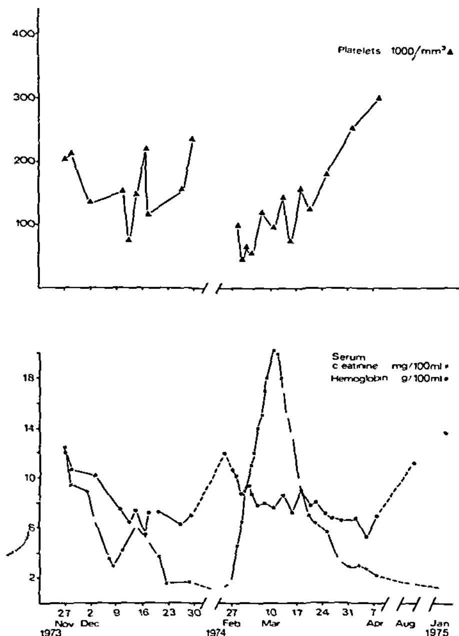


Fig 1 Course of renal function and blood changes in a case of antazoline induced renal failure hemolysis and thrombocytopenia

plement activity measured as hemolytic activity was normal The C4 factor measured by Mancini technique (11) was lowered while the C3 factor was normal (Table I) Bone marrow examination showed a normoblastic but reduced erythropoesis and the iron stores were good The myeloic cells showed signs of toxic effects Blood eosinophilia reached a maximum of 33 % In serum there were no cold agglutinins no antinuclear factors and a normal protein spectrum Agarose electrophoresis of the urine showed tubular proteinuria and no M-component

Renal biopsy on Dec 13 Light microscopy showed no cell proliferation or membranous changes in the glomeruli The tubular epithelium was irregularly flattened and contained some chromoprotein casts The interstitial tissue of cortex and outer medulla was moderately infiltrated by lymphocytes and eosinophilic cells and there was a slight edema There was not sufficient material for immunofluorescence examination Blood was sampled for investigation of drug allergy

The patient received no other drug than promethazine (Lergigan®) during the following weeks On Jan 4 1974 she was discharged from hospital and at controls at the Outpatient Clinic her serum creatinine decreased to 1 2 mg/100 ml and Hb increased to 12 1 g/100 ml

On Feb 25 the patient took 2 tablets of a salicylate against headache Two days later she noticed edema of her face and since she suspected an allergic reaction she took a tablet of antazoline (Antasten®) a drug she was used to

tak ng n such s tuat ons Some hours later she developed abdom nal pain nausea vomit ng ch lls bloody urine and was taken to the hosp tal She had a Qu ncke edema and fever 39 5°C BP 1 0/90 mmHg Abdom nal status was normal no spleen enlargement On this adm ss on her serum was found to be red and her urine was red even after centrifugation Spectrophotometric exam nation of the urine disclosed methemoglob n The urinary sed ment contained only 3–4 erythrocytes per h gh power f eld Her sclerae were sl ghtly icteric and serum bil rub n was ? 7 mg/100 ml SGOT and SGPT were sl ghtly elevated On the following day she was anuric and had d arrhoea She was trea ed with high doses of cort sone furosem de a res n and bicarbonate The serum creatinine rose to a maximum of 19 mg/100 ml and urea N to 128 mmol/l The d ures s ncreased during the f rst few days to 600 ml/d but she reached the polyuric phase only after 10 days Total complement activity as well as the C4 factor were lowered while the C3 factor was normal (Table I) Hb decreased f om 11 0 to 7 0 g/100 ml there was a trans ent leucocytos s of ?3 600/mm³ a thrombocytopenia of 56 000 and re iculocytos s of 3 6% There was eos nopen a (due to the cort sone treatment) Haptoglobin 0 5 g/l ESR reached a max mum of 160 mm Bone marrow exam nat on showed ncreased erythropoes s and reduced ron stores Antazol ne was now most suspected of be ng the offend ng drug but the immunological analyses d d in lude other suspected substances

The second hosp talization lasted for 6 weeks the serum c eat nine be ng ? 2 mg/100 ml at d scharge The recovery was slow after the last attack of acute renal failure and the normalization of laboratory values has taken several months (F g 1)

IMMUNOLOGICAL METHODS

Complement quan at on and complemen consumpt on test

Total serum complement amount was measured as hemolytic act vity Dilut ons of pa ent serum (1/1 1/? 1/4 and so on) were incubated w th sheep blood cells sens tized wi h an sheep blood antibod es as described by Lachmann et al (7) After ncubat on at 37°C for one hour and at 4 C for 1? hours the hemolys s was registered The h ghest d lu tion w th 100% hemolys s was noted A pat ent serum was def ned to have one complement unit f the d lut on of 1/8 gave 100% hemolys s Thus poss ble complement un ts were <0 ?5 0 25 0 5 1 0 ? 0 3 0 and so on Heal hy nd v duals in general have 1 3 units The amount of com p ement factors C3 and C4 respectively was determ ned by the Manc ni method (11) (Table I) us ng Part gen® plates (supplied by Behringwerke AG Marburg Lahn) Normal range of C3 is 110–170 mg/100 ml and of C4 it s 12–37 mg/100 ml

Complement consumpt on in v tro was tested by add ng the substance to be tested to pa ent serum taken three mon hs after the last Antasten® exposu e and ncuba ng the mixture at 37°C for 45 m n After ncubat on complement quantitat on was performed The negat ve con rols were buffered sal ne in ead of the tested substance as well as serum from heal hy nd v duals nstead f p ent serum

The follow ng s bsta ices were tes ed (he end concen

Table I *Complen ent ; t t o*

Date	Total complement act v ty (U)	C? fa (mg/ l
Dec 1? 1973	1 0	110
Feb ?7 1974	<0 25	9?
March 8 1974	? 0	137
March 9 1974	3 0	118

trat ons in the m xture are nd a ed w h n p e Antasten® (antazol nechloride) (0 50 0 05 0 0 Lergigan® (promethaz nechloride) (5 m Doctac ll n® (amp c ll n) (6 30 0 μg m e (pentobarb tal) (5 10 0 μg/ml) a e s l al cy a 0 5 0 05 mg/ml)

An asten® antibody determ nation

Ant bod es against Antasten® we e d te m enzyme l nked immunosorbent a a (El orig nally described by Engvall et al 3 Bo IgM and IgE ant bod es were measu ed anti IgA and ant IgM from Dakopa s A S D anti IgE from Pharmacia AB Sweden

Coombs tests were performed as described b and Gell (?)

Results of immunolog cal anal ses

Complement consumpt on test n vitro showe Antasten® but not the other drugs caused a p on decrease n the pat ent s serum complement act v The h ghest concen rat on of Antasten® (0 50 mg m e creased serum complement to an unde ectable level No change was seen when Antasten® was added to normal serum There was also a decrease n C4 concen a on when the largest amount of Antasten® wa added wh e C3 was not affected at all

The ELISA method gave a sl ghtly increased level of IgG ant bod es against Antasten® when compared to blood donors No other Antasten® ant bod es were seen.

Ind rect Coombs test us ng normal red blood cells (human ORh-) in the presen e of Antasten® and pa ent serum taken three months af er the last exposure to Antasten® was pos t ve

DISCUSSION

Th s case report illustrates a rare cause of acute renal fa lure The attack of flank pain wh ch in tiated the sequence of events has not been expla ned but after three episodes of acute renal failure it was poss ble to single out the culprit of these ep sodes To some extent it was also poss ble to elucidate the mechanisms of hemolys s and renal damage There is only one earl er report on immune hemolysis and renal fa lure caused by antazol ne (9) That case

seemed clinically to be similar to ours but there was no association with thrombocytopenia and the renal lesion was not discussed Furthermore we could also show antibodies to antazoline as well as complement consumption

The IVP caused an anaphylactic shock Mild allergic manifestations due to X ray contrast media are not uncommon but severe reactions are rare when using the modern triiodized media (5) On admission to hospital 2 weeks later it seemed reasonable to regard the renal failure as shock induced tubular necrosis Since the BP fall had been of short duration a co-factor might have been suspected The finding of macroscopic hematuria was not verified by microscopy but the finding of microscopic hematuria 2 weeks later seemed to confirm the report from the outpatient clinic

The second episode of acute renal failure was preceded by a pentobarbital induced urticaria It is probable but not clear that the patient took an antazoline tablet during a home leave On this second occasion allergic mechanisms were strongly suspected to be the direct cause of renal damage A pronounced anemia also developed but the tests for hemolysis were not conclusive The bone marrow smear showed no increase in the erythropoiesis but rather signs of toxic changes This finding is compatible with the severe renal impairment during the preceding weeks and thus the bone marrow could t respond to a hemolytic stimulus The macro pic hematuria was again rapidly transient t the absence of even microscopic hematuria 2 days later should have aroused suspicion as to the cause of the red urine

The investigation of drug allergy was not yet complete when the patient got the third and most severe attack of acute renal failure On this occasion there was a full blown picture of hemolysis massive hemoglobinuria and renal failure with anuria She was on the verge of needing dialysis treatment Antazoline was the only common denominator for the first and third episodes and probably also for the second

It has been recognized for the last two decades that drugs can provoke antibody formation which may lead to destruction of the patient s red cells (6) The destruction is caused by an antigen antibody reaction taking place on the surface of the red cells in the presence of complement This is the allergic response classified by Coombs and Gell (2) as the type 2 reaction which can be diagnosed by the in vitro reaction e g agglutination of antigen-coated red cells by patient serum a reaction which can be enhanched by addition of anti immunoglobulin serum There are various hypotheses to account for the exact mechanism of this drug induced immune reaction and the most favored one seems to be that the antigen consists of drug + red cells in union The number of drugs which provoke this type of reaction is very limited In a recent survey of all reported cases (14) 16 drugs were listed and except for penicillin each of these drugs was represented only by 1–4 cases

The drug dependent Coombs test in our patient indicates that the hemolysis was of the immune hemolytic type referred to above the type 2 reaction The negative or only weakly positive direct Coombs test using the patient s own red blood cells taken a few days after exposure to antazoline mi be explained by a rapid elimination and inactivati of antazoline

Our patient developed thrombocytopenia paral with the hemolysis and most convincingly during t third episode It might well be speculated that t thrombocytopenia was due to a similar immune rea tion as the hemolysis Some of the drugs reported cause immune hemolytic anemia are more prone induce immune thrombocytopenia e g quinine a quinidine It is rare however to find thromb cytopenia associated with immune hemoly anemia (14) and there are only two such cas reported one induced by quinidine (4) and one phenacetin the case of MacGibbon et al (8) capitulated by Worlledge (14) A case of thromb cytopenic purpura caused by sensitization to ant zoline has been reported (13) The transient leuc cytosis is compatible with the hemolytic disord

The light microscopy of the renal tissue obtain on the third day of the second episode showed t picture of tubular necrosis undergoing regeneratio and diffuse interstitial nephritis with infiltrates lymphocytes and eosinophilic cells

In eight of the patients reported with drug induce immune hemolytic anemia there was evidence renal failure and some of them even died fro uremia Nevertheless the renal damage was hardl commented upon and the probable mechanism renal involvement was not discussed It has bee experimentally shown that Hb by itself does n produce renal lesions (10) and mechanical obstru tion by crystals of the pigment is not a cause of anur (12) There is no relation between the intensity of th

hemolysis and the seriousness of the renal disorder The combination with some other factor such as circulatory insufficiency or severe dehydration is necessary to produce renal lesions These factors are discussed in the textbooks of renal disease and they imply that the renal failure is a complication of hemolysis and hemoglobinuria However the renal cells may also be damaged directly as the result of an immune reaction as suggested by MacGibbon et al (8)

In our case the mechanism behind the first episode of acute renal failure might have been two or threefold shock hemoglobinuria and immune reaction The biopsy taken one month later pointed to a tubular epithelium undergoing regeneration On the second and especially on the third occasion it seemed more plausible that an antigen antibody formation causing complement activation was responsible for the development of renal lesion Thus the renal lesion might be due to a type 3 reaction according to Coombs and Gell (2) The results of the ELISA analysis showing antazoline antibodies of IgG type as well as the complement consumption shown both in vitro and in vivo strengthen the assumption that a type 3 reaction was involved The histopathology of the renal tissue could also be compatible with an allergic nephropathy most frequently described in methicillin hypersensitivity (1) Unfortunately the specimen for immunofluorescent studies was inadequate

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Drug-induced Febrile Mucocutaneous Syndrome

With a Survey of the Literature

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ABSTRACT A retrospective investigation of 89 cases of the Stevens Johnson and/or Lyell's syndrome showed that 77 patients (87%) had been treated with drugs, 2/3 of them for symptoms of respiratory and/or urinary infections The most frequent drugs were longterm sulfonamide preparations, penicillins, butazones and antiepileptics Mycoplasma pneumoniae and Staphylococcus aureus were the most common infective agents of those reported More than 40% of the patients were below 20 years of age There were more women than men The mortality was 1-2% for the Stevens Johnson syndrome and 4 of 11 patients for Lyell s syndrome It must be pointed out that, in the individual patient, it can be difficult to determine the etiology, especially in cases with infections

It is considered that the febrile mucocutaneous syndrome (ectodermosis pluriorificialis syndroma Stevens Johnson) and toxic epidermal necrolysis (Lyell s syndrome) can be caused by drugs and/or infections (14)

In order to throw light on drugs as a cause of the disorder as well as an increase in its incidence seasonal variations etc a material with the diagnoses Stevens Johnson and Lyell s syndromes has been analysed retrospectively The cases are among those reported to the Swedish Adverse Drug Reaction Committee supplemented with cases from the National Board of Health and Welfare s patient statistics from the Uppsala region

MATERIAL

All cases of Stevens Johnson syndrome erythema exudativum multiforme mucocutaneous syndrome and epidermal necrolysis reported to the *Adverse Drug Reaction Committee* in the period 1966-72 were scrutinized Forty five patients met the criteria exanthema fever and involvement of at least two orifices Extensive desquamation had been noted in 9 patients largely complying with the criteria for Lyell s syndrome As there seem to be transitional forms between these two syndromes the entire material of 54 patients was analysed together (see Discussion)

In the *hospitals in the Uppsala region* (population 1 2 mill) all diagnoses at discharge are registered in a computer (19) The case reports with the diagnosis ectodermosis pluriorificialis (syndroma Stevens Johnson) from the years 1964-69 were requisitioned and studied The criteria for the Stevens Johnson syndrome were met in 39 cases and Lyell s syndrome in 3 In 12 of the case records there was no indication that the patient had taken any drug Of the 30 patients who had received drugs prior to their skin and mucosal symptoms 7 had been reported to the Adverse Drug Reaction Committee and are thus included in the group above The combined material placed at our disposal by the physicians accordingly comprises 77 patients who had received drugs and 12 who probably had not

RESULTS

The distribution by age and sex is given in Table I In both groups 42% of the patients were younger than 20 years Women predominated in the group receiving drugs men in the much smaller no drug group Clinical symptoms of respiratory and/or urinary infections were present in 2/3 of the patients at the onset of the illness whereas no infection was found in the remaining 1/3 (Table II) Information about the infective agent had been recorded for 18 patients in the drug group (Table III) 6 of them had *Mycoplasma infection* and 4 Staphylococcus aureus infection In the group one patient presented with aureus and one with Pneumococc

Table I *Age and sex distribution of the patients*

Age	Drug group		No-drug group	
0–9	8	42%	4	42%
10–19	24		1	
20–29	13		2	
30–39	5		1	
40–49	11		1	
50–59	6		2	
60–69	7		1	
70–79	2			
80–89	1			
Men	22	29%	9	
Women	55	71%	3	
Total	77		12	

The drugs which had been administered before the onset of the skin and mucosal changes are listed in Table IV Sulfonamides had been given to 40 patients 32 of whom had received preparations with long term effect (The group other sulfonamide preparations+another drug includes one case after sulfadimethoxine) Forms of penicillin derivatives of butyl phenyl pyrazolidine (phenylbutazone etc) and antiepileptics had been given to 13, 12 and 5 patients respectively The remaining seven patients had received six different preparations between them

An allergic disposition (hereditary or a previous history) had been noted for 24 patients in the drug group and denied by 23 no information about this available for the other 30 Concurrent disorders any other factors that may have contributed are rheumatoid arthritis and food in three cases each unspecified collagen disorder psoriasis exposure to sun and menstruation in one case each

The annual incidence in the Uppsala region has been 5–10 cases/mill with little variation from year to year The drug induced cases have accounted for

Table II *Clinical symptoms of infection at the time of onset*

Site of infection	Drug group		No-drug group
Airways	26	71%	7
Urinary tract	26		0
Other	6		1
None	24	29%	4
Total	82[a]		12

[a] Five patients had more than one type of infection

Table III *Infective agent at the time of onset*

	Drug group
Mycoplasma	5
Mycoplasma + influenza A	1
Staphylococcus aureus	4
Herpes simplex	2
Mononucleosis	1
α streptococci	1
β streptococci	1
Proteus mirabilis	1
Tularemia	1
Pseudomonas aeruginosa	1
Total	18

a constant proportion (approximately 70%) of the total number

The skin–mucosa reaction proved fatal for 3 patients in the drug group 4 of them had Lyell s syndrome The changes healed without complication in 62 patients The complications in the other 10 were impaired vision ($n=3$) hepatic effects ($n=2$) and loss of nails There were no deaths or complications in the no drug group but the changes recurred in four patients

In the material from the Uppsala region drugs had been given to 30 patients but not to the other 12 according to the case records Of the 30 patients in whom the skin and mucosal changes had been preceded by drugs 7 were reported to the Adverse Drug Reaction Committee—a report rate of 20%

DISCUSSION

The *febrile mucocutaneous syndrome* (ectodermosis pluriorificialis syndroma Stevens Johnson etc) is characterized by fever impaired general condition exanthema and involvement of mucosal membranes usually in the mouth and eyes but sometimes also around the nostrils and the anal and genital region It has been pointed out that the exanthema is multiform and exudative in only 1/3 of cases in the others it may be maculopapular hemorrhagic morbilliform or scarlatiniform (21) The degree of inflammatory changes in the body orifices varies and the changes may be membranous or ulcerous

Toxic epidermal necrolysis (Lyell s syndrome scaled skin syndrome etc) can be included in the febrile mucocutaneous syndrome in the wide sense of this term but displays certain peculiarities The skin changes are initially erythematous but become

Table IV *Drugs suspected*

	No of pats*	
Sulfonamides		40 (3)
Sulfamethizole + sulfamethoxy pyridazine (Sulfapral®)	16 (2)	
Sulfamethizole + sulfamethoxy pyridazine + other drug	5 (1)	
Sulfamethoxydiazine	10	
Other sulfonamide preparations + another drug	9	
Penicillins	2	13
Ampicillin	2	
Ampicillin + other drug	2	
Other penicillins	7	
Other penicillins + other drug	2	
Pyrazolon derivatives		12 (1)
Phenylbutazone or oxyphenbutazone	9	
Phenylbutazone or oxyphenbutazone + other drug	3 (1)	
Antiepileptics		5
Carbamazepine	4	
Primidone	1	
Other drugs		7 (1)
Belladonna extract + phenobarbital	1	
Salicylates	2	
Chlorzoxazone acid acetylsalicyl dextropropoxiphenchloride (Paraflex comp®) + paracetamol chlormezanone (Lobac®)	1	
Sodium aurothiosulphate	1 (1)	
Methyldopa	1	
ACO children s ointment and powder	1	
Total		77 (5)

No of deaths within parentheses

bullous and the skin is shed in large flakes exposing the corium Nickolsky s sign is positive Superficial mucosal membranes may be involved The general status is affected and mortality is high (27) The two syndromes have different histopathology and prognosis but cases of Stevens Johnson syndrome have been known to turn into Lyell s syndrome (14) In view of the difficulty in distinguishing between them retrospectively they are here analysed together

Causes

Causes of the Stevens Johnson syndrome are listed by Rook et al (14) as drugs infections diseases of the connective tissue malignancy pregnancy and contact reactions those of Lyell s syndrome are reported to be drugs and Staphylococcus infections

Drugs

Several drugs have been reported to elicit the *Stevens Johnson syndrome* e g penicillin sulfonamides phenolphthalein antiepileptics barbiturates pyrazolone derivatives and acetylsalicylic acid (1 9 22) Sulfonamide preparations with a long term action have been discussed in particular Carroll et al (4) presented 116 cases of the Stevens Johnson syndrome after such preparations from the years 1957–65 Having criticised this report for a lack of clinical data and information about other conceivable precipitating factors Bianchine et al (1) presented figures from three medical centres showing that there had been no significant change in frequency of admissions of patients with the Stevens Johnson syndrome after the introduction of long term sulfonamides in 1957 Their total series comprised 138 cases from the period 1952–65 24 patients had not received drugs and 66 had been treated for symptoms which could have been precursors of the Stevens Johnson syndrome or innoccuous respiratory and urinary infections The proportions of no drugs respiratory and/or urinary infection and no infections were largely the same as in the present material In their material long term sulfonamides were associated with the Stevens Johnson syndrome less frequently than were penicillin short term sulfonamides phenolphthalein and sedatives

In a survey of the literature Bianchine et al (1) found 426 cases of the Stevens Johnson syndrome 59% drug induced and 41% caused by infection or of unknown origin The long term sulfonamides featured most frequently among the drug induced cases even when the 116 patients of Carroll et al (4) were excluded Next came short term sulfonamide preparations antiepileptics and penicillins

A material of 638 skin reactions in the period 1961–70 from the Department of Dermatology at Helsinki University Hospital (9) included 16 cases of the Stevens Johnson syndrome and 36 of Lyell s syndrome Sulfonamides had induced 11 of the former 9 had received sulfamethoxypyridazine alone or as a combined drug The other drugs involved were barbiturates and meprobamate + acetylsalicylic acid One case after a peroral contraceptive has been reported (12)

In a critical survey of the literature on the Stevens Johnson syndrome Monnat (11) found that

42% of 176 patients had taken drugs prior to its onset 38 had received sulfonamides (12 of them sulfamethoxypyridazine) 27 antibiotics 12 pyrazolone derivatives 10 salicylates and 9 barbiturates (some had taken more than one drug)

Of the 36 cases of *Lyell s syndrome* reported by Kauppinen (9) 24 had received sulfonamides 20 of them had taken sulfamethoxypyridazine alone or as a combined drug The others had been given phenylbutazone derivatives antiepileptics and in one case each carbamazepine and chloramphenicol+acetylsalicylic acid

Monnat s study included 195 patients with Lyell s syndrome of whom 80% had taken drugs before its onset antibiotics in 78 cases sulfonamides in 54 (long term in 22) and pyrazolone derivatives in 53

Hunez et al (8) presented 23 cases of their own and cited a dissertation by one of the co-workers covering 533 cases of Lyell s syndrome In the latter material the predominant drugs were sulfonamides (46%) particularly those with a long term action (39%) penicillins were involved in 20% (mostly procaine penicillin) hypnotics antiepileptics and antiphlogistics as a group in 25% The mortality was high in the early reports 50% but subsequently fell to 25–17%

A group of 15 cases of Lyell s syndrome reported by Björnberg (2) included 5 after sulfamethizole+sulfamethoxypyridazine (Sulfapral®) after penicillin and 1 after phenylbutazone le cases have been published in recent years long term sulfonamides (5 13 29) aminoone phenylbutazone (10) barbiturates (28) nicillin (3) and pentazocine (7) These reviews overlap to some extent but prompt the same conclusions 80–90% of Lyell s syndrome and 40–80% of the Stevens Johnson syndrome have occurred in conjunction with the intake of drugs the most common being sulfonamides particularly the long term variety antibiotics pyrazolone derivatives antiepileptics and barbiturates

Sulfonamides (52%) predominated in the present material chiefly the combination sulfamethizole+sulfamethoxypyridazine (Sulfapral®) and sulfamethoxydiazine After them came penicillins butazones and antiepileptics

Infections

Infection alone or combined with drugs has been connected with the onset of the Stevens Johnson syndrome The precipitating agents are reported to be mycoplasm (17 23) adenovirus herpes simplex hemolytic streptococci salmonellosis mononucleosis infectiosa epidemic hepatitis vaccinia and variola (24 25 26)

It is held that phage type 71 staphylococci can induce Lyell s syndrome particularly in children (18 20) though occasional cases have been reported in adults (6 15) According to Rothenberg et al (15) the reactions induced by staphylococci or by drugs can be distinguished histopathologically the staphylococci form an exotoxin with affinity for stratum granulosum the cells are split off from underlying viable epidermis In other forms of Lyell s syndrome there is degeneration of basal cells with spongiosis and necrosis of the entire epidermis resulting in separation near the dermoepidermal border It has been shown by direct immunofluorescence that immunoglobulin and complement are located to the basal-cell layer

In the present material symptoms of infection were present in 2/3 of the patients Mycoplasms were found in 6 patients and herpes simplex in 2 A boy of 7 months with Lyell s syndrome had staphylococci in cultures from nose throat and skin but no clinical signs of infection on admission to hospital Before the symptoms appeared he had been treated with a children s ointment and powder which at that time (1964) contained boric acid But although boric acid can induce toxic epidermal necrolysis (16) the most probable cause of the present case was the staphylococcal infection In another three (adult) patients with marked flaking of the skin there was a secondary occurrence of staphylococcus aureus in the skin in two and in the blood in one The no drug group included one case with a staphylococcal infection and one with pneumococci

Although cutaneous reactions of the type discussed here can be elicited by infections it is drugs which predominate as the aetiological factor in this and earlier materials

There is no definite seasonal or annual variation in the incidence of cases in the material reported to the Adverse Drug Reaction Committee or in that from the Uppsala region The drug group comprises about 70% women and 30% men the small no-drug group roughly the opposite Men accounted for about 60% of the Stevens Johnson cases presented by Bianchine et al (1) and by Monnat (11) and for 54% of Monnat s cases of Lyell s

syndrome In keep ng with earlier reports the present patients were mostly young—4?% younger than ?0

The mortal ty in the Stevens Johnson syndrome is reported to l e between 5 and 15% in an untreated material (14) though Carroll (4) arrived at a f gure of 25% One of the present patients d ed i e 1 3% In the case of Lyell s syndrome Rook et al (14) state that the prognos s depends on the aet ology being better in ch ldren when the reaction is induced by staphylococci than whe i the agent is a drug The mortality in the literature referred to above ranges from 8 to 52% In the present material 4 of 11 pat ents died

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The First Two Cases of Coccidioidomycosis in Finland

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ABSTRACT Endemic areas of coccidioidomycosis are described The mycology of Coccidioides immitis *is reviewed, with special reference to the risk of* laboratory infection A classification of coccidioidomycosis is listed and two cases from Finland are presented The first patient had typical X ray and clinical findings, including a positive coccidioidin test The second case, occurring soon after a visit to Arizona, was confirmed by positive fungal cultures from the sputum and from a resected specimen Because the latter patient became pregnant and the risk of dissemination was considered great legal abortion was performed The resection of the pulmonary cavitation with infiltration around it took place under coverage of pre and postoperative amphotericin B medication Complete recovery was obtained The indications for thoracic surgery are briefly listed Diagnostic and mycologic aspects of this rare and exotic disease are discussed Among other things, the importance of a good medical history in finding the contact with infective environment or material is stressed

Coccidioidomycosis is a dust borne fungal disease which is endemic in arid areas of South western United States (San Joaquin Valley in California Arizona New Mexico and Texas) and in northern parts of Mexico There are some reports from South and Central America too A few cases have been reported from Scandinavia usually imported from endemic areas by visitors Sometimes the infection results from laboratory exposure to Coccidioides immitis

When grown as the mycelial form in soil or in cultures outside the body the light and highly infectious arthrospores spread easily in the air The parasitic form (the spherules) in man is not infectious Therefore the disease is not considered communicable from man to man

Clinically coccidioidomycosis is quite like tuberculosis first a usually benign and self limited primary infection which leads to lasting immunity The other form is a post primary reinfection which spreads hematogenously and can have various severe progressive clinical features

Outside the endemic areas suspicion of coccidioidomycosis can arise only if two premises are fulfilled Firstly the examining doctor must have knowledge of this rare and exotic disease Secondly the patient must have had a clear contact with Coccidioides spores either in an endemic area or on exposure to laboratory cultures or to other infective materials

CLINICAL FINDINGS

The main clinical features of coccidioidomycosis and the pathological changes associated with each of these forms are classified as follows

Primary coccidioidomycosis A) Asymptomatic disease with subclinical infection which becomes evident from conversion of coccidioidin test to positive *B)* Symptomatic cases with febrile respiratory illness *(a)* With pulmonary changes including infiltrations cavitations hilar lymphadenitis or pleuritis *(b)* Including in 5% of cases hypersensitivity phenomena as erythema nodosum arthritis or eye symptoms

Residual pulmonary lesion Solid coccidioidomas thick or thin walled cavitation(s) fibrotic or calcified end point lesion(s)

Disseminating coccidioidomycosis of hematogenous origin or by direct extension *(a)* Respiratory (lung bronchi pleura thoracic wall) *(b)* Systemic involving one or more organs or tissues e g lymphatic tissue skin eye meninges all visceral organs and skeletal bones

Concerning the various symptoms and other diagnostic problems the reader is referred to textbooks and previous papers (11 13 15 18)

CASE REPORTS

We present here two patients seen in our clinic who to our knowledge were the first two cases of coccidioidosis



Fg 1 (a) A round dense shadow n the m ddle lobe *(b)* A th n walled cav tat on in the same locat on n the right lung and small dens t es n the left lung

ed in F nland A descript on of these cases s con to be of value because of the numerous nterest ng typ cal features nvolved and because t may ass st n recogniz ng any new cases n F nland and other Scand na vian countries outs de the endem c areas

Case 1

The pat ent was a F nn sh tra ned nurse born in 1930 who had worked n London s East End n 1952–53 tak ng care of healthy and pol o ch ldren In 1954 a round shadow was seen in the right lung but gradually d sappeared spontane ously Two years later an egg s zed ound dense shadow was observed n the left h lar region close to the ma n bronchus The infiltration decreased w th n about a year but n 1957 a c rcular shadow 0 5 cm n d ameter ap peared n the apicoposterior segment of the upper lobe In 1958 another round shadow was seen lower n the left lung and a round dense shadow about 1 cm n d ameter n the right middle lobe The latter dens ty became empty in 1959 forming a th n walled cav tat on By the follow ng year more small c rcular shadows had appeared espe cially in the left lung (F g 1)

The pat ent was tubercul n pos t ve (Mantoux 1 10000) but the d sease d d not respond to tuberculos s drugs (SM INH PAS) The sta n ng and culture were perfo med a number of t mes always w th negat ve results When t became ev dent that the disease was not tuberculos s other exam nat ons were made and among other results a pos t ve cocc d o d n sk n test was obtained (1 1000 1 100 strong pos t ve) On later control the test wa s 1 more strongly pos t ve but serological tests gave negat ve react ons The only add t onal s gn f cant f nd ng n laboratory tests was eos noph ha w thout leukocy os s 11% and 17% of the total WBC Spu um cultures y elded only a saprophytic stra n of Asperg llus

Based on the cl n cal features sk n test pos t ty and neffect veness of over 7 yea s of tuberculos s therapy a d agnos s of cocc d o domycos s pulmonum amborum was arrived at Later the cond t on became qu escent w hout therapy earl er the symptoms had been very sl ght con s sting of las tude and occas onal fever The pa ent moved to Canada n 1961 and a spec al st s sta ement requested from England by the Imm grat on Off ce ga e a d agnos s of cocc d o domycos s When last contacted 10 years after em grat on the pat ent was n good heal h



F g 2 A th n walled coccid o dal cav ty in the left upper lobe at the time of first adm ssion



F g 3 The walls of the cav ty became th cker and a soft infiltration appeared peripheral to the cavity near the pleura

Case 2

A young woman born in 1949 vis ted Southwest Un ted States in 1968–69 staying for about a year w h her sister s fam ly n Tucson Arizona where the cl mate s and w th air-borne desert dust and ra nfall only two or three t mes a year She also went rid ng in the desert a number of times Tw ce she was ill w th what was bel eved to be tons ll s for which because of pen c ll n allergy she rece ved some other ant'b ot c

Thorax X ray n Jan 1970 showed a ca ty shadow in the upper part of the left lung The pat ent was tubercul n negat ve to 1 T U and 10 T U She refused sanatorium treatment and was g ven a tetracycl n therapy as out-pat ent It had no effect and the s tuat on was kept under observat on The X ray finding decreased n s ze and had nea ly d sappeared n a control exam nat on made over two years later In Sept 197? there was onset of a severe non productive cough and two days later of fever up to 39 C During tetracycl n treatment t subsided w th n about a week to a subfebrile level but the cough pers sted Thorax X ray now showed at the s te of the earl er infiltra t on a plum s zed very clearly c rcumscribed cav tat on w th very d st nct boundaries and the pat ent was sent to the Department of Pulmonary Diseases of the Hels nki Un vers ty Central Hosp tal with the d agnosis coc cid o domycos s? Tb?

On admiss on n the latter part of Oct 197? she was n a good cond on w th a sl ght subfebrile even ng tempera ture as the only symptom In thorax X ray a fairly thin walled av ty shadow 18×30 mm n s ze was seen n the ap coposterior segment of the left lung (F g ?) The sta n ngs and cultures were negat ve but the cocc d o d n sk n test was pos tive after 48 hours (d ameter 8 mm) H stoplasmin test was negative The complement f xat on test was pos t ve (1 ? overn ght ++) as well as the im



Fig 4 Coccidioides immitis 14 days after inoculation Rectangular arthrospores and pieces of hyphae (phase contrast ×750)

munodiffusion test (Dr D Pappagianis University of California School of Medicine) Fungus culture from sputum yielded Coccidioides immitis (A Kahanpaa)

While waiting for the final results of the above tests the patient was allowed to stay at home since she was fairly free from symptoms On returning to hospital she was pregnant despite previous warnings and a decision of legal abortion (aap) was made because of the coccidioidomycosis This was performed in mid December 1972 After this there was radiological progression of the disease The walls of the cavity in the left lung became ... cker and small soft infiltration patches developed ... the cavity interpreted as extension of the infection

... ring dissemination resection of the lesion in the left ... g was considered to be indicated preceded by amphotericin B medication Starting late in Dec 1972 she received amphotericin B (Fungizone®) in increasing daily doses of 2 500 5 000 10 000 20 000 40 000 and 50 000 IU (50 000 IU = 50 mg) but administration was suspended for a time because of a fall in the creatinine level to 137 μmol/l Later doses of 50 000 IU every other day caused headache and nausea regardless of antihistamine administration creatinine rose again to 188 μmol/l and a number of pauses were necessary The total preoperative dosage of amphotericin B was 500 mg

On Jan 26 1973 thoracotomy and resection of the left upper lobe were performed in the clinic for chest surgery Concordantly with the X-ray findings the apicoposterior segment of the left upper lobe contained a firm mass the size of an egg and filled with gray granular pus Culture yielded Coccidioides immitis fungus The histologic picture was that of necrotizing granulomatous inflammation a finding considered to fit tuberculosis or a fungal infection e g coccidioidomycosis

Recovery from thoracotomy was good and the patient received postoperative amphotericin B medication in the Department of Pulmonary Diseases Side-effects appeared again and a total dosage of 450 mg was attained of the target of 500 mg Creatinine was elevated to 150 μmol/l necessitating pauses in the medication

At discharge one month after operation the patient was feeling well though the lung was not fully expanded to fill the thoracic cavity She has been in good health and in early 1974 gave birth to a healthy child She is attending check-ups semiannually

MYCOLOGIC FINDINGS

In direct microscopy no fungus particles were seen in either untreated or Gram stained sputum specimens (Nov 1 1972) Fungus cultures were made of sputum on nine culture media (10) Growth of yeast appeared on some media within two or three days (Candida albicans) On the fifth day after inoculation quite abundant fungus growth was seen on Kimmig g agar (contains gentamycin) at 37°C and similar growth was also observed on the following day among the bacterial growth on Kimmig agar at 30°C Similar pale gray fuzzy growth with a slightly yellowish background was not visible on other culture media during six weeks culture Microscopic examination of the fungus growth 14 days after inoculation showed among other findings a fairly large amount of arthrospores (Fig 4) and a part of the mycelia were racquet hyphae The fungus was identified as Coccidioides immitis Since this is a very rare microfungus in Finland the strain was sent to Centralbureau voor Schimmelcultures in the Netherlands where Dr G A de Vries reached the same conclusion

Confirmation of this strain as Coccidioides immitis was also obtained in animal experiments At the Department of Serology and Bacteriology of the University of Helsinki where all the mycologic examinations were made unless otherwise stated the strain was injected into guinea pigs on Nov 24 1972 Dissection on Nov 30 revealed definite signs of inflammation in the scrotum Direct microscopy did not show fungus particles in the exudate but fungus culture of the latter yielded within six days at 30°C on Sabouraud agar and at 37°C on Kimmig-g agar and Sabouraud sp agar (contains streptomycin and penicillin) an abundant grayish white fuzzy fungal growth which proved to be Coccidioides immitis From the Netherlands the following statement was obtained on the animal experiments carried out with the original strain isolated from sputum The culture has produced granulomata in the lungs and livers of intraperitoneally inoculated laboratory mice In the liver ripe spherules were seen with many endospores in the lungs the spherules were not yet ripe

Fungus cultures as described above were also made from the resected pulmonary cavitation on Jan 26 1973 Growth of Coccidioides immitis appeared on Kimmig and Kimmig-g agars and very scanty growth on malt agar

DISCUSSION

From the European aspect coccidioidomycosis is an exotic disease the occurrence of which in North

Europe has been promoted by increasing tourism multinational armies foreign trade etc transit through an endemic area may also suffice for acquisition of the disease (9 11 13) Several animal species (dog horse sheep etc) can contract coccidioidomycosis while the cat apparently is resistant to it At least one case of canine infection has been published from Scandinavia (14)

Laboratory personnel handling cultures of Coccidioides in the arthrospore phase can readily become infected by the respiratory route unless adequate precautions are taken (7 17) Coccidioides immitis is a biphasic filamentous fungus that can be cultured from clinical material or from soil on a number of fungus culture media Thin membranous foci may appear already within three or four days soon followed by a growth of white fuzzy mycelia which later turn grayish (as in case 2) or brownish In microscopic examination there are seen developing on the hyphae chains of more or less rectangular arthrospores about 2–4 μ in size (Fig 4) which easily break into individual fragments These minute arthrospores can become dispersed in the air and if inhaled are capable of penetrating into the lungs as far as the alveolar level In tissues they increase in size and become spherules about 10–80 μ in diameter that give rise to endospores of about 2–5 μ

According to the series studied in 1950 by Elo and Patiala (5) skin tests with both coccidioidin and histoplasmin usually give a negative reaction in Finnish subjects whereas in endemic areas chiefly as a result of subclinical disease up to several tens of percents of the population may give a positive reaction (13) Our case 2 was the first positive one among 400 persons tested at the Department of Skin Diseases of the Helsinki University Central Hospital (8) Accordingly the coccidiodin test and information on previous foreign travel can be taken as appropriate points of departure in considering the probability of coccidioidomycosis in an inhabitant of a non-endemic area Patient 2 had stayed for some time in an endemic area in Arizona whereas the source of infection could not be determined in patient 1 possibly she had in some way come into contact with infective material during her stay in London As said both patients gave a definitely positive reaction in the coccidioidin test but only in case 2 was the complement fixation test positive In her case confirmation of the diagnosis was very convincing mycologically (from sputum specimen and resected lung tissue) as well as serologically It should be pointed out that by no means all cases of coccidioidomycosis give a positive finding in sputum culture and that growth may be very scanty (12 17) Diagnosis in case 1 was based more on the very typical X ray finding and on the exclusion of tuberculosis

Coccidioidomycosis has sometimes a rather unpredictable and fitful course Besides maintenance of the patient s general condition treatment consists mainly of i v amphotericin B but this is a highly toxic substance (1 3 4 9) Since spontaneous recovery is also possible as can be concluded from the various stages in cases 1 and 2 it is not always easy to evaluate the need of treatment In cases of disseminated coccidioidomycosis however chemotherapy is considered to be almost always indicated In untreated cases the outcome is fatal in about 50% (3 4 18) Residual pulmonary coccidioidomycosis is a form with usually a more favorable prognosis though the risk of dissemination must be born in mind In this form the residual pulmonary cavitations usually disappear with amphotericin B often within a few months At present the total mortality from coccidioidomycosis is generally a few percent (6)

In pulmonary coccidioidomycosis it may occasionally be necessary to resort to thoracotomy for the differential diagnosis especially to exclude carcinoma Among the indications for lung resection in confirmed cases of coccidioidomycosis are persistent hemoptysis recurrent cavity and enlarging peripheral cavity or destroyed lobe or lung Pregnancy is a further indication for thoracic surgery as in our case 2 The risk of dissemination in association with thoracotomy is not very great especially if done under coverage of pre and post operative amphotericin B medication (12 16) which became possible in case 2 after performance of aap

As is often the case with aspergilloma (2) it appears to be the fate of patients with coccidioidomycosis particularly in non-endemic areas to be first treated for pulmonary tuberculosis in view of the similarity of the general symptoms and the lung X ray finding (12 15) This happened in our case 1 even though there was early suspicion of a more rare disease possibly a non European mycosis Even with mycologic assistance from abroad identification of the disease proved difficult Ten years later when another patient with

coccidioidomycosis came to the same Department of Pulmonary Diseases of the Helsinki University Central Hospital progress in diagnosis and previous experience contributed to the establishment of the correct diagnosis with much greater directness thoroughness and ease

The occurrence of sporadic cases of coccidioidomycosis in Scandinavia is possible and the attending physician must therefore be able to suspect this disease particularly in persons who have visited an endemic area It should also be kept in mind that pulmonary tuberculosis and coccidioidomycosis may occasionally be concomitant (11 12 16)

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BOOK REVIEWS

The lives of a cell By Lewis Thomas 153 pages The Viking Press Publishers New York 1974

The lives of a cell is a surprising title on a stimulating book with many other surprises Why plural? It seems clear that each cell has only one life The theme of the book however is to show how many separate units of life exist inside one cell membrane The author compares the cell with an organism and also describes stacks of individuals—the anthill—as a complete body or why not a complete cell? Many pages of the book are full of paradoxes and several chapters are difficult to accept on first view This makes the reading exciting and the presentation of the facts from all fields of biology is crystal clear even if the conditions are complex and unusual The book challenges rereading

The notion that a cell has its own organelles performing service as integrated parts of the whole just as bees and ants are members of a community—a sort of superindividual unity—this is nothing new in itself The examples are taken from all fields of biology but perhaps chiefly from the world of microorganisms where Lewis Thomas feels very much at home after many years in the borderland between clinical medicine and microbiology Some of the concepts are shocking to a doctor who is used to regard scientific development as a continuous series of discoveries proving the suitability of biological regulations The author tells us about the recent important discoveries regarding the use of the horseshoe crab (Limulus polyphemus) a living fossile that has stayed the same for some 200 million years in spite of or perhaps because of the fact that it reacts in an unusual way to endotoxins As a matter of fact its blood is now used as the most sensitive indicator of these deadly poisons that are so important in medicine This reaction may be lethal to the whole animal and Thomas concludes that this may become a mechanism of overkill we are in effect at the mercy of our own Pentagons most of the time!' You may call this phenomenon suicide by defense—there are several in human medicine and more will be discovered

During the last years Lewis Thomas has become a medical statesman whose overview of the big field is used in an advising capacity An excellent chapter from the realm of medical organization has the title Your very good health He asks the question if the recent trend to computerize medicine is taking us in the right direction or whether this will turn out to be a house of IBM cards He puts the question bluntly if we are having a lot of luxury consumption of health care and states the important truth (possibly going to some millions crowns) that most things get better by themselves This has only been realized by a few enlightened doctors and their families who live according to the motto Most things in fact are better by the morning Vis medicatrix naturae is a strong force if we dare to put our faith in it And many recent remedies carry risks!

The book is not only full of problems and paradoxes *it is a well of wisdom that should be read* by the medical profession—and also by biologists who are interested in medicine as a branch of biology

Jan G Waldenstrom

Contrasting concepts of ischaemic heart disease By Gunnar Biorck 138 pages Sw cr 59 65 Almqvist & Wiksell International Stockholm 1975

Gunnar Biorck is without any doubt the master mind in Swedish medical journalism when he prescribes what he has himself aptly called Medicine for the politicians His many articles in daily newspapers and semimedical journals have influenced both politicians and electorate during the last decades and started violent and healthy discussions

This time the author has collected his ideas on one of the most crucial problems of

medicine the mortality from coronary heart disease (CHD) Above all prevention but also different aspects of acute and continued treatment of the victims of CHD is something that must interest all members of a modern Western society and the last part of the volume has the title What to tell our statesmen about ischaemic heart disease?

The volume contains the two Eli Lilly lectures held by the author in November 1974 The first was presented in Oxford the second at the Royal College of Physicians in London The style is easy as we are used to find it from this author The presentation is scientific even if it is fluent and makes good reading for everybody The first part of the book title contains the words contrasting concepts and the author gives the contrast between the different scientific schools and perhaps still more between different philosophies of life It is refreshing to notice that the author does not only represent an active school of modern invasive or non invasive cardiology but also the art of practicing medicine among sick individuals He writes—quoting an imaginary statesman musing to himself (and for the author?) Perhaps I should have a check up made? Or should I not? What is the best to know or not to know? What do I want with my own life? To live on or to accomplish what I have set before me? The eternal questions condensed by the Romans in the famous words on a tomb stone *vixit dum vixit laetus* (he lived happy as long as he lived) are discussed by the author in a stimulating way

The first lecture treats somatic non philosophical problems even if the author does not—happily—avoid stating his own subjective ideas Both lectures together give a very important picture of CHD in three countries with different patterns Finland high incidence England medium and Sweden low These questions are discussed on the basis of numerous schematic drawings and graphs specially prepared for these lectures Studies during the last two decades by Biorck and his group at the Seraphimer Hospital in Stockholm have illustrated many of the most important facts of CHD and are reviewed and discussed in detail It has given an impressive and massive contribution to our knowledge

The author quotes Osler when he said that it is the duty of a medical department to tabulate and analyze carefully recorded experience Biorck has done this in an excellent way but he—like Osler—has gone much further He has integrated all his data in his own mind and presented the results in such a lucid form that anybody may have a personal opinion—critical or approving—as the case may always be in important matters The book may be read with great profit by all persons who have a responsibility for the two sides of medicine health care and healing

Jan G Waldenstrom

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Liver, Lung and Malignant Disease in Heterozygous (Pi MZ) α_1-antitrypsin Deficiency

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ABSTRACT The occurrence of PAS positive, diastase resistant, inclusion bodies in hepatocytes has been used as a marker of heterozygous (Pi MZ) α_1 antitrypsin deficiency in order to study the frequency of lung liver and malignant disease in a consecutive autopsy material from a well defined population Typical PAS positive inclusion bodies were found in the liver in 26 (3 7%) of the 700 cases studied In these 26 subjects both liver (cirrhosis or fibrosis) and lung disease (emphysema) were significantly more prevalent ($p<0\ 001$) than among PAS negative controls. The prevalence of malignant disease did not differ between the groups The results are consistent with the concept that heterozygous (Pi MZ) α_1 antitrypsin deficiency predisposes to both liver and lung disease but does not influence the survival rate

Twenty four molecular variants of α_1 antitrypsin the main protease inhibitor (Pi) in serum have been described in man Of these variants only severe α_1 antitrypsin deficiency (Pi ZZ) has unequivocally been shown to be associated with early emphysema (6) and with liver disease in children (22) and adults (2) Heterozygous (Pi MZ) α_1 antitrypsin deficiency with approximately 60% of the normal serum α_1 antitrypsin level, occurs with a frequency of 3–4% (6 9) in the Scandinavian countries

The question whether Pi MZ individuals are predisposed to chronic lung disease is controversial several investigators (14 17) claim to have found an increased prevalence of such disease among carriers of the Z allele but others (23 24) have reached an opposite conclusion Subclinical abnormalities of lung mechanisms however seem to be common in such individuals (13) The role of other Pi phenotypes with intermediate serum α_1 antitrypsin levels (Pi SS SZ MS) in the pathogenesis of chronic obstructive lung disease is still more uncertain as no large materials have been studied A characteristic finding in Pi ZZ individuals and in carriers of the Z gene is an accumulation of PAS positive diastase resistant globular hyalin bodies within the hepatic cells (21) These bodies consist of a large aggregate of an asialo antitrypsin in a dilated endoplasmic reticulum (8 11) They occur in Pi ZZ individuals irrespective of the presence or absence of liver disease (12) and though less abundantly also within hepatocytes of MZ individuals Case reports describing cirrhosis in carriers of a single Z gene have appeared (3 5) and an increased prevalence of malignant hepatoma in such individuals has been suggested (2 20) Recently a study was published suggesting no increased prevalence of MZ individuals in cryptogenic cirrhosis (19)

The present study was undertaken firstly to investigate the prevalence of lung (emphysema) liver (fibrosis or cirrhosis) and malignant disease in a large autopsy material in which the occurrence of PAS positive inclusion bodies in the liver was used as a marker of α_1 antitrypsin deficiency of the Z phenotype and secondly to study the effect of these complications on the survival rate

MATERIAL AND METHODS

At the Department of Pathology General Hospital Malmo Sweden 75% of all individuals dying in Malmo (270 000 inhabitants) are autopsied Each year about 1 500 autopsies are performed in a standardized manner including complete micro- and macroscopic reports of most organs The present study is based on the first 700 consecutive autopsies in 1973 Complete autopsy records were available

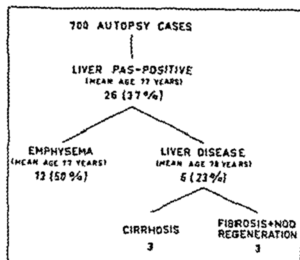


Fig 1 Main findings in the autopsy material Liver PAS positive indicates the occurrence of PAS positive inclusion bodies after diastase treatment

Besides samples for conventional histological examination two liver samples (each approx 2×1×0 5 cm one from the right and one from the left lobe) were taken from all subjects PAS staining was performed on sections from the liver samples before and after diastase digestion The sections were examined by several independent observers Liver disease was judged from the same sections stained with hematoxylin-erythrosin and van Gieson s staining for collagen fibers Lung disease (e g emphysema) was judged solely from the macroscopic appearance of ie lungs at autopsy The first 100 consecutive autopsied without PAS positive bodies within the hepa were used as controls A clinical analysis was all available records

RESULTS

Fig 1 and Table I summarize the main findings Twenty six subjects (3 7%) showed typical PAS-positive inclusion bodies Of these 13 (50%) had macroscopic emphysema and 6 (23%) cirrhosis ($n=3$) or fibrosis ($n=3$) with nodular regeneration The frequency of lung and liver disease was significantly higher ($p<0\ 001$) among PAS positive individuals Among the PAS-negative controls cirrhosis was found in one and fibrosis with a tendency to nodular regeneration in another subject (Table I)

The PAS positive patients with confirmed cirrhosis were non alcoholics All had signs of portal hypertension at autopsy The diagnosis of cirrhosis had been made ante mortem in two clear emphysema also diagnosed ante mortem was present in two The three PAS positive subjects with fibrosis and nodular regeneration had no portal hypertension but in two of them low prothrombin levels and jaundice had been noted ante mortem Emphysema was not seen in any of these cases Most subjects ($n=20$) with PAS positive inclusion bodies had an otherwise normal liver The bodies were observed in various parts of the hepatic lobules but most regularly in the periportal area (Fig 2)

Emphysema had been recorded at autopsy in 50% of the PAS positive patients compared with 18% in the control group, a highly significant difference (Table I) In 5 of the 13 subjects with emphysema and hepatic globules the diagnosis of emphysema had been made during life and in 2 additional cases chest X rays showed signs of emphysema Of the emphysema patients 5 were cigarette smokers but 4 were non smokers In the remaining cases notes about smoking were not available

The prevalence of malignant growths did not differ significantly between the two groups (Table I) and no case of hepatoma was found in the PAS positive group

The survival rate of PAS positive subjects and PAS negative controls did not differ The mean age at death was 76 and 74 years respectively an insignificant difference (Table I)

Pre mortal blood samples were available from two PAS positive subjects with cirrhosis Pi typing

Table I *Prevalence of emphysema cirrhosis or fibrosis and malignant tumours in 26 patients with PAS positive inclusion bodies and in 100 PAS negative controls*

	Mean age (y)	Emphysema	Cirrhosis or fibrosis	Malignancy
PAS-positive	76	13	6	14
PAS negative	74	18	2	41
	$p>0\ 1$	$p<0\ 001$	$p<0\ 002$	$p>0\ 1$



Fig 2 Liver with no morphologic abnormalities except for slight steatosis PAS-positive globules are distributed throughout the hepatic lobule PAS stain after diastase digestion ×484 In the right lower corner magnification ×1200

(15) showed MZ phenotype in both In the third PAS positive patient with cirrhosis agarose gel electrophoresis excluded the Pi ZZ phenotype

Agarose gel electrophoresis had been performed in only 7 patients with PAS positive inclusion bodies The relative levels of acute phase reactants in them are shown in Fig 3 and compared with the levels in PAS negative controls The α_1 antitrypsin level was subnormal in most PAS positive cases in spite of high levels of other acute phase reactants a pattern compatible with the MZ phenotype

DISCUSSION

In the past three years numerous studies have shown that individuals carrying the Pi Z gene have PAS positive inclusions in the liver (4) This phenomenon seems to be pathognomonic of the Z gene deficiency and has not been described in normal individuals with the Pi MM phenotype or in association with any other phenotype within the Pi system (1 12 19) The cellular structures within the liver in the Pi SS phenotype with reduced α_1-antitrypsin level have been described as normal (12) and no hepatocellular abnormality has been observed in the rare Pi 00 phenotype (10) Thus the subjects in this study with PAS positive inclusion within their hepatocytes in all probability represent the MZ phenotype Furthermore the finding of 3 7% PAS positive livers in a series of 700 individuals is in good agreement with the expected Pi MZ frequency in Sweden (4%) (6) In addition the acute phase reactant pattern found in 7 PAS positive subjects strongly supports the concept that these represent the MZ phenotype and the two PAS positive subjects who had been tested during life were in fact MZ The chances of Pi ZZ individuals having been included seem negligible since the frequency of homozygotes (Pi ZZ) in Sweden is only 1/1 500 (6) and the globules in the hepatocytes of such individuals are as a rule much more abundant (4)

A basic defect in individuals carrying the Z gene is an inability of the hepatocytes to secrete an insoluble asialo-α_1 antitrypsin which is aggregated in the endoplasmic reticulum (8) Hypothetically it may be possible for a Pi MM individual to accumulate simi

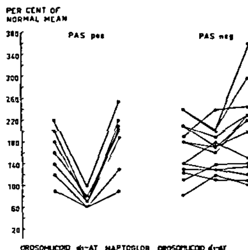


Fig 3 Acute phase reactants trypsin and haptoglobin) in PAS-posi compared to PAS negative controls T are given as a percentage of the plasma (from 1000 healthy donors) patients α_1-antitrypsin is nd

lar material in the liver during e g a massive infection but no such cases have been reported Judging from our own and others experience no MZ individuals lack PAS-positive inclusions in their hepatocytes provided a large biopsy is inspected But inclusions may easily be overlooked in ordinary needle biopsies due to the small sample size An examination of 700 liver biopsies from the routine files in this hospital revealed PAS positive bodies in only 1 % of the cases

The high frequency of emphysema (50 %) in PAS positive individuals is of interest in view of the conflicting reports on the prevalence of such disease in intermediate deficiency states (13) There is no reason to believe that the pathologists had over estimated the frequency of emphysema in the PAS positive subjects as they were unaware of the patient s Pi type at the time of autopsy Our data support the concept that Pi MZ individuals are prone to develop emphysema and are in remarkable agreement with the prediction of Mittman et al (18) that every second MZ individual develops emphysema after 50 years of age

It was not possible to assess the role of cigarettes in this series because so many records lacked notes about smoking habits Like the situation in our series with cirrhosis in adult Pi ZZ individuals (7) emphysema had escaped detection intra vitam in a gh proportion of cases

r data also suggest an increased prevalence of osis and fibrosis of the liver in Pi MZ individu s (Table I) Among Pi ZZ subjects no more than 10 % will develop cirrhosis in adult life and thus constitute a small part of all non alcoholic cirrhosis cases (7) Our finding of three subjects with non alcoholic cirrhosis and portal hypertension in a group of 26 PAS-positive patients as compared with 1 of 100 PAS negative controls is significant because of the high frequency (4 %) of MZ individuals in the population and because it provides a new quantitatively important aspect of liver disease in α_1 antitrypsin deficiency In a recent report (19) on the frequency of the MZ phenotype in a series of patients with cryptogenic cirrhosis no association could be established but lack of information about mean age of the patients rules out a comparison with their material As in Pi ZZ adults (7) the cirrhosis appears late in life and portal hypertension and complications do not seem to occur before the age of 60–70 It is also evident that in spite of the high frequency of both emphysema and liver disease in PAS positive patients their survival rate is not reduced (Table I)

Our data do not suggest any relation between antitrypsin deficiency and a predisposition to various types of malignancy (Table I) We have previously reported a high frequency of malignant hepatoma in Pi ZZ individuals with cirrhosis (7) and suggested a relation between hepatoma and MZ phenotype (2) Two MZ patients with hepatocellular carcinoma have recently been reported by Rawlings et al (20) No hepatomas were found in the present series Though this absence does not warrant any definite conclusions the results do suggest that this complication is probably due to the presence of a cirrhosis and not directly to the metabolic error per se

From a clinical point of view this study suggests that Pi typing should be performed liberally in adults especially in old patients with cryptogenic liver disease The finding of heterozygous (MZ) α_1 antitrypsin deficiency should direct the physician s attention to the possibility of concomitant chronic obstructive lung disease especially emphysema

ACKNOWLEDGEMENT

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The Risk of Atherosclerotic Vascular Disease in Subjects with Xanthomatosis

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ABSTRACT The morbidity and mortality in 172 males and 164 females with xanthomatosis have been investigated Symptoms of coronary heart disease (CHD) were the most frequent initial manifestation of atherosclerotic vascular disease Angina pectoris was the first symptom in about 3/4 of males as well as females myocardial infarction was the first event in 26% of the males and 9% of the females Other manifestations of atherosclerosis were comparatively rare and occurred late in life Half of the subjects were affected with symptoms of atherosclerotic vascular disease by the age of 52 in men and 62 in women, the mean age for first symptoms being 49 and 56 years, respectively No significant influence of other CHD risk factors than xanthomatosis and hyperlipidaemia was found in these patients An increase in the number of cardiovascular deaths was seen in xanthomatosis patients, compared with the general population, in particular in the number of ' sudden deaths ' Half of the males died before the age of 66 and half of the females before the age of 74 5, which is about 10 and 7 years earlier than predicted at 30 years of age for the normal population The cumulative relative mortality in both men and women was about twice that expected for the general Norwegian population until 70 years of age

The risk of contracting coronary heart disease (CHD) increases with rising plasma cholesterol and triglyceride concentrations (3 9 19) Individuals with hereditary hyperlipoproteinaemia therefore have an increased risk of atherosclerosis and its complications The size of this risk in relationship to that in the general population has not been fully established Xanthomatosis is a sign of significant lipid deposits in tissues and presumably represents a relatively severe lipid disorder

This study attempts to estimate the risk of atherosclerotic disease and the significance of certain clinical findings in an unselected series of subjects with xanthomatosis

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MATERIAL AND METHODS

Subjects

The probands were persons with xanthomatosis Most but not all of these subjects had hyperlipoproteinaemia Type IIA lipoprotein pattern was most common in the patients with hyperlipoproteinaemia although type IIB and type IV patterns were also found Further details about the lipid and lipoprotein pattern in the subjects with xanthomatosis have been reported (7)

The subjects with xanthomatosis were classified into three groups probands dead probands and secondary cases the last group consisting of affected relatives of persons belonging to one of the other groups This investigation includes data on 172 males and 164 females all with xanthomatosis

Diagnostic criteria

Atherosclerotic vascular disease was diagnosed if CHD intermittent claudication or cerebral atherosclerosis were present CHD was diagnosed if angina pectoris and/or myocardial infarction were present or had been experienced

Myocardial infarction This diagnosis was accepted when two of the following three criteria were fulfilled 1) Typical clinical history 2) Serial changes in ECG suggesting or proving myocardial necrosis (Q waves) or injury (ST elevations) 3) Transient increase in one of the following serum enzymes aspartate transaminase (ASAT) creatine phosphokinase (CPK) lactic dehydrogenase (LDH) or hydroxybuturic dehydrogenase (HBDH) There was good agreement between the hospital diagnosis and the diagnosis arrived at when these criteria were applied

Angina pectoris Angina pectoris was usually from the patient s history but often

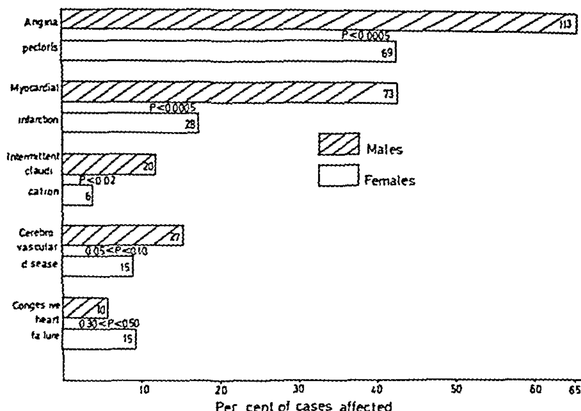


Fig 4 Percentage of males and females with xanthomatosis showing different manifestations of atherosclerotic vascular disease The absolute numbers of affected subjects in each group are given at the end of each bar and the *p* values given are for the differences between males and females for each diagnostic category

The manifestation of atherosclerotic disease at time of investigation The different manifesta 's of atherosclerotic disease which were present t the time of investigation (or at death) are given in Fig 4 More than one manifestation was commonly present and males were more frequently affected than were females Congestive heart failure was more frequent among females than males

The distribution and numbers of myocardial infarctions experienced among the different patient categories are given in Table V

Causes of death

The causes of death among patients with xanthomatosis are listed in Table VI If the group sudden death is taken to represent cardiovascular

Table V *Proportion of subjects with myocardial infarction in each patient group and the mean number of myocardial infarctions in those affected*

	Males				Females			
	Probands	Dead probands	Secondary cases	Total	Probands	Dead probands	Secondary cases	Total
No of subjects with myocardial infarction/ no of persons in group	35/80	17/23	19/69	71/172	10/70	6/12	12/82	28/164
Mean no of myocardial infarctions in those affected	1 4	1 7	1 3	1 45	2 1	1 5	1 3	1 64

Table VI *Causes of death among xanthomatosis patients*

	Myocardial infarction	Sudden death	Chronic congestive heart failure	Cerebro-vascular disease	Rupture of abd aorta	Other	Total no of deaths
Males							
Probands	4	11	3		1	2	21
Dead probands	9	10	1	1	1	1	23
Secondary cases	9	6	2	1		3	21
Total	22	27	6	2	2	6	65
Females							
Probands	4	3	1	1			9
Dead probands	3	5	3			1	12
Secondary cases	9	9	1	1			20
Total	16	17	5	2		1	41

events 91% of deceased men and 98% of deceased women died from such causes The groups myocardial infarction and sudden death together account for 75 and 80% of the deaths in males and females respectively The three causes of death ischaemic heart disease sudden death and cerebrovascular disease among the patients with xanthomatosis and among the general population in the area of study (Østfold county) have been compared in Table VII Although ischaemic heart disease was more frequent than expected in both male and female patients ($\chi^2=2\ 77$ 1 d f $p\sim0\ 10$ for males $\chi^2=11\ 95$ 1 d f $p<0\ 001$ for females) the largest difference is in the proportion of sudden deaths ($p<0\ 0001$ for both males and females) Deaths from cerebrovascular disease were less frequent in xanthomatosis patients than in the general population ($p<0\ 01$ for males $p<0\ 02$ for females) No age correction for mortality was used in these calculations When the general pattern of causes of death in Østfold was compared with that in Norway as a whole no systematic deviation was found in the diagnostic trend

Mortality

The cumulative probability of death with increasing age in subjects with xanthomatosis is shown in Figs 5 6 and 7 based on life tables as illustrated in Table VIII The group of dead probands had a greater probability of death in both males and females than had the other subjects at the same age The mortality was higher at all ages in men than in women and for all patients compared with the rates in the Norwegian males and females respectively Half of the male and female subjects with xanthomatosis died before the age of 66 and 74 5 years respectively The life expectancy in persons with xanthomatosis at 30 years of age is 10 years shorter for males and 7 years shorter for females than in the general population

Table VII *Comparison between the causes of death in patients with xanthomatosis and in the general population (~220 000 inhabitants) in Østfold county (no adjustment for age)*

	Total no of deaths	Causes of death: Ischaemic heart disease	Sudden death	Cerebrovascular disease	Total
Population in Østfold					
Males	1 265	409 (32 3%)	46 (3 6%)	209 (16 5%)	664 (52 5%)
Females	1 025	262 (25 3%)	22 (2 1%)	229 (22 3%)	513 (50 0%)
Patients with xanthomatosis					
Males	65	28 (43 1%)	27 (41 5%)	2 (3 1%)	57 (87 7%)
Females	41	21 (51 2%)	17 (41 5%)	2 (4 8%)	40 (97 6%)

Table IX. *Blood pressure in xanthomatosis patients on whom sufficient information was available in relation to atherosclerotic vascular disease and death*

	No. of individuals with BP level			Total no. of individuals	*p*
	Normal	Borderline	Increased		
Males	132	18	11	161	<0.01
Females	107	23	29	159	
Angina pectoris					
Affected	123	24	27	174	N.S.
Unaffected	116	17	12	145	
Myocardial infarction					
Affected	68	15	14	97	N.S.
Unaffected	169	26	24	219	
Cerebrovascular disease					
Affected	24	8	9	41	<0.05
Unaffected	214	33	39	277	
Intermittent claudication					
Affected	16	4	6	26	N.S.
Unaffected	219	37	32	288	
Congestive heart failure					
Affected	14	5	6	25	N.S.
Unaffected	228	36	34	298	
Dead	65	12	17	94	N.S.
Alive	174	29	23	226	N.S.

N.S. = not significant

frequency between males and females was not significant. The frequency of systolic murmurs in[illegible]sed with age. The true frequency of aortic [illegible]is is unknown.

[illegible]rneal (lipoid) arcus. A corneal arcus was found [illegible]2% of the males and 84.4% of the females examined (no ophthalmologic examination was performed). No significant differences were found between different groups of patients.

The presence of a corneal arcus was correlated with age and to a certain extent with lipid levels. Those below 50 years of age without a corneal arcus had cholesterol concentrations below the mean and a strongly developed arcus in a young subject generally reflected relatively high cholesterol values.

Relation between ECG patterns and the presence of CHD. ECG recordings of satisfactory quality (alternating currents were often disturbing) were obtained in 100 (72%) of 139 subjects without symptoms of CHD. The pattern at rest was judged as

Table X. *Atherosclerotic disease in xanthoma patients in relation to smoking*

	With no information	Non-smokers*	Ex smokers	Smokers	Total
Males					
Affected	46	14	19	44	123
Unaffected	20	5	3	21	49
Total	66	19	22	65	172
Females					
Affected	39	28	4	6	77
Unaffected	26	47	1	13	87
Total	65	75	5	19	164

* Less than 5 g/day

Table XI *Body parameters (mean±S D) in different categories of patients with xanthomatosis*

	No of subj	Height (cm)	Weight (kg)	Relative weight (%)
Males				
Cholesterol normal	58	174 7±5 5	75 4±11 2	106 4±12 4
Cholesterol increased	70	175 5±7 5	74 0±10 4	105 4±12 0
Females				
Cholesterol normal	50	163 7±5 4	67 4±13 0	110 3±19 9
Cholesterol increased	83	162 6±6 4	62 2±11 4	102 7±19 0
Males				
Triglycerides normal	99	175 6±6.5	74 6±11 0	105 0±12 1
Triglycerides increased	29	173 5±6 9	75 8±10 0	108 6±12 1
Females				
Triglycerides normal	90	163 2±10 1	63 4±11 2	103 6±20 4
Triglycerides increased	43	156 7±7 7	65 3±13 1	109 7±17 4

normal in 90 cases and pathological in 10 cases Two persons with normal resting ECG had abnormal patterns after exercise

Similarly ECGs were obtained in 167 (84 4%) of 197 subjects with symptoms of CHD 2/3 of them had pathologic recordings at rest After moderate exercise 2 more (out of 17) exhibited pathologic patterns The frequency of pathologic patterns increased strongly with age

DISCUSSION

Patients with xanthomatosis have been considered at high risk of CHD since Muller (14) pointed out that xanthomatosis hypercholesterolaemia and CHD (with early death) form a hereditary syndrome The present investigation of a relatively unselected population with xanthomatosis confirms this but considerable variability in the clinical course was seen Although the subjects were heterogeneous with respect to lipid disturbances the great majority fulfils the criteria for type II hyperlipoproteinaemia according to Fredrickson and Levy (7)

The difference in ascertainment between probands and secondary cases could possibly reflect differences in the clinical course between the two groups This was not borne out however in morbidity and mortality rates when life tables were constructed and the apparent differences were due to age differences Only minor differences were found in the lipid and lipoprotein pattern between the probands and the secondary cases (7)

Both men and women in the groups of dead probands had an earlier onset of disease and died younger than did the probands and secondary cases and they therefore represent a selected group with respect to these parameters With this exception a considerable uniformity with respect to morbidity and mortality between the groups was found for each sex Males had onset of atherosclerotic vascular disease and died earlier than did females The time at which half the males and females were dead is shortened by about 10 and 7 years respectively from that expected for Norwegians at 30 years of age

The age at the time of onset of symptoms was related to the lipid concentrations as subjects with a maximal cholesterol value of 500 mg/100 ml and higher had their first symptoms earlier than patients with lower values Normolipidaemic subjects appeared to have a lower risk than those who had high values of both cholesterol and triglycerides However high lipid values hypertension cigarette smoking or overweight were not associated with a significantly increased mortality but the number of deaths in each group was small Hypertension cigarette smoking and overweight appear to be overshadowed by the lipid disorder as risk factors in these patients

Treatment did not seem to reduce mortality but the series studied were not suited for analysis of effect of treatment on survival The group was clearly selected since the treated subjects had earlier onset and more severe symptoms than the untreated

The sites of predilection for atherosclerosis as

Table XII *Probability (%) of symptoms of CHD and death at different ages in the present xanthoma patients compared with subjects with type II hyperlipoproteinaemia reported in three other investigations*

	Males						Females							
	CHD			Death			CHD				Death			
Age (y)	40	50	60	50	60	70	40	50	60	70	40	50	60	70
Present investigation	13	43	83	13	31	68	0	20	45	72	0	3	11	39
Stone et al (18)*	16	33	52				8	20	32					
Jensen et al (8)	19	45		19	29	76	25	75			5	18	50	56
Slack (17)	24	51	85	23	54	78	0	12	58	74	0	0	15	15

* Only subjects with myocardial infarction or death from myocardial infarction are included

judged from the symptoms were the coronary arteries as almost all subjects had symptoms of CHD as the initial manifestation of vascular disease The use of a standardized questionnaire for angina pectoris would have made the data more comparable with other investigations but was not undertaken The systematic use of exercise ECGs in those without cardiac symptoms would probably have further increased the rate of affection but only symptomatic affection was scored

Symptoms of atherosclerotic vascular disease in the legs and in the cerebral vessels were comparatively rare and occurred late in life The mortality in cerebrovascular disease was lower in xanthoma patients than in the general population Athero-[illegible]lerosis is prominent in the cerebral vessels in [illegible]terolaemic subjects (4) but death from [illegible] may occur before the cerebrovascular [illegible] give symptoms This opinion is not shared by de Gennes et al (5) who found symptoms of cerebrovascular disease in particular from the basilaris area in more than 50% of their subjects with xanthomatosis However their criteria for affection were wider than those applied here

The causes of death were predominantly cardiovascular and although myocardial infarction was more frequent and appeared earlier than in the normal population the greatest difference compared to the general population was found in the proportion of sudden deaths

The estimated prognosis with regard to morbidity and mortality of CHD is comparable to that given by other authors (8 17 18) for those affected with type II hyperlipoproteinaemia (Table XII) The numbers of affected and dead subjects vary considerably in those series as do the diagnostic criteria (when stated) The largest is the NIH series (18) in which the morbidity and mortality in a selected series of subjects with type II hyperlipoproteinaemia were compared with those in their unaffected relatives Affected males contract their illness and die about 10 years earlier than do females Otherwise morbidity and mortality patterns are similar in both sexes Harlan et al (6) found in their series a mean age at the onset of CHD of 42 years in males, compared to 45 years in five other papers summarized by these authors The mean age at manifestation of vascular atherosclerotic disease in the present series was 49 years in males and 56 years in females corresponding figures for females being 50 and 53 years respectively in their own series and in the series summarized by them

Half of the males and females had died within 8 years of onset of atherosclerotic disease manifestations and these figures are comparable to data from the Framingham investigation (10) Angina pectoris was the initial manifestation more frequently in women than in men in the present investigation

A corneal arcus was an early sign of hyperlipoproteinaemia and was found in the great majority of subjects it was missing only in young or normolipidaemic individuals In the present series the frequency of corneal arcus was clearly higher than that reported by de Gennes et al (5) in their series of patients with xanthomatosis and also higher in all ages than that reported for the same area (1)

Females with normal triglyceride values were significantly taller than females with increased triglyceride concentrations in the present series This finding is in contrast to that reported by Miettinen and Aro (13) who found that the hypertriglyceridaemic subjects were taller and heavier than those with hypercholesterolaemia The same authors also reported a higher frequency of aortic

systolic murmurs than that found in this series (62% versus 24%)

A tendency to very early death was noted in some families in the present study This observation was not limited only to families with greatly elevated plasma lipid concentrations Exceptions to a familial pattern of early death were seen in certain females For instance one female proband had had no sign of CHD until the ripe age of 91 years whereafter she developed a moderate angina pectoris and a severe cardiac neurosis upon the discovery that she had hypercholesterolaemia with a maximal value of 520 mg/100 ml She died suddenly two years later Such cases should perhaps give second thoughts about drastic therapeutic measures in families where longevity is the rule (at least in females) It is noteworthy that half of the females in this series could expect to reach an age higher than 74 5 years

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Lp(a) Lipoprotein and Pre-β_1-Lipoprotein in Relation to Lipid Levels in Males

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ABSTRACT Previous studies have shown that a slow moving pre β-lipoprotein fraction, named the pre β_1 lipoprotein occurred significantly more frequently among subjects with coronary heart disease (CHD) than among healthy individuals This lipoprotein is closely related to, and probably identical with, the Lp(a) lipoprotein Immunological tests likewise showed that Lp(a) lipoprotein was significantly more common among patients with CHD than among controls Mean cholesterol and triglyceride levels were higher in pre β_1 lipoprotein positive than in pre β_1 lipoprotein negative individuals Lp(a+) individuals tended to have higher serum cholesterol values than did Lp(a–) persons but there was no difference in the mean triglyceride value This apparent discrepancy seems to be due to the presence in occasional sera of lipoprotein fractions with pre β_1 mobility, usually of a VLDL nature These lipoproteins are not associated with the Lp(a) lipoprotein

An epidemiological study in Northern Sweden disclosed a positive association ($p < 0.001$) between the occurrence of an atypical slow moving pre β lipoprotein fraction and angina of effort (12) This fraction which was called pre β_1 lipoprotein exhibited several properties suggesting a close relationship with the lipoprotein carrying the genetically determined Lp(a) antigen (1 2 3) The latter lipoprotein known as the Lp(a) lipoprotein has a density of 1 050–1 080 g/ml

Several studies performed independently and blindly (4 8 9) demonstrated a highly significant positive association between the presence of Lp(a) antigen and the occurrence of pre β_1 lipoprotein upon electrophoresis of serum in 0 5% agarose The results were compatible with the view that immunological Lp(a) testing with the standardized technique used is more sensitive than agarose gel electrophoresis in detecting the Lp(a) lipoprotein

Lp(a) antigen and pre β_1 lipoprotein were found significantly more frequently among subjects with coronary heart disease (CHD) than among healthy people (4 8) However some samples which exhibited a pre β_1 lipoprotein fraction upon electrophoresis were typed as Lp(a−)

In several studies (4 7 8 10 11 the mean values for total cholesterol and triglycerides were higher in individuals with an electrophoretically demonstrable pre β_1 lipoprotein than in those without This was the case whether cellulose acetate (Sepraphore III) membranes (10 11) or 0 5% agarose (8 11) was used for the lipoprotein electrophoresis However as the pre β_1 lipoprotein classification differed somewhat between the two electrophoretic methods (11) agarose electrophoresis became the method of choice

In a series of 100 Finnish patients with suspected or proven CHD (13) the mean cholesterol value was found to be 248 mg/100 ml in the Lp(a+) group and 228 in the Lp(a−) group The difference was of borderline significance A previous study (4) had shown a non significant trend towards a higher cholesterol value in Lp(a+) than in Lp(a−) persons but no suggestion of a difference with respect to triglyceride values In the same study we found that one Lp(a−) person whose serum exhibited a lipoprotein component with pre β_1 mobility upon electrophoresis had a very high serum concentration of triglyceride This finding suggested that lipoproteins characterized by very low density (VLDL) may occasionally cause problems in the electrophoretic determination of pre β_1 lipoprotein We now report further studies on this problem and

Table I *Pre β_1-lipoprotein and Lp(a) antigen in 107 50–52 year-old Swedish males*

	Pre β_1-lipoprotein		
	Present	Absent	Total
Lp(a+)	18	10	28
Lp(a−)	7	72	79
Total	25	82	107

χ^2=32 44 1 d f p<0 0001

additional analyses of the relationship between serum lipid levels and Lp(a) lipoprotein/pre β_1 lipoprotein

MATERIAL

Sera from 107 Swedish males aged 50–52 were investigated These persons were drawn from a population subsample of 157 presumably healthy middle aged men Several variables in 103 of the 107 men have been reported (8)

METHODS

Blood was drawn in the morning after a 12 hour fast Following centrifugation serum was submitted to lipo-electrophoresis in 0 5% agarose and a portion of sample was shipped in the frozen state by air to the , Oslo where immunological Lp typing (1 2) done Triglyceride assay was conducted by the method (6) Total and free cholesterol were determined by gas chromatography (11)

Serum samples exhibiting a pre β_1-lipoprotein fraction upon electrophoresis were submitted to preparative ultracentrifugation at the density 1 050 g/ml for 16 hours at 114 000×g using conventional techniques Following ultracentrifugation the top layer and the bottom layer were separated to secure fractions with a density lower and higher than 1 050 g/ml respectively These fractions were submitted to electrophoresis in 0.5% agarose

Six persons were bled again and the serum was submitted to preparative ultracentrifugation this time including a run at the density 1 006 g/ml (14)

RESULTS

Test for association between Lp(a) antigen and pre β_1-lipoprotein

Table I shows the distribution of the 107 males with respect to Lp phenotype and presence or absence of pre β_1 lipoprotein A highly significant positive correlation was found between the two lipoprotein phenomena (p<0 0001)

Density characteristics of electrophoretically demonstrable pre β_1-lipoprotein

Following ultracentrifugation of the 25 sera which exhibited a pre β_1 lipoprotein fraction upon electrophoresis it could be shown that this lipoprotein component resided in the density class >1 050 g/ml in 19 of the sera and in the density class <1 050 in the remaining 6 When new samples from these 6 persons were studied it was found that the lipoprotein component with pre β_1 mobility resided in the density class <1 006 g/ml in 4 of them whereas no pre β_1-fraction was demonstrable in the remaining 2 No component with pre β_1 mobility was demonstrable in fractions with a density higher than 1 006 g/ml from any of the 6 sera

It was concluded that the density of lipoproteins with pre β_1 mobility is usually higher than 1 050 g/ml but that exceptions occur Fig 1 shows pre β_1 lipoprotein in normal sera and in the lipoprotein density class >1 050 g/ml

Relationship between Lp(a) antigen and pre β_1-lipoprotein components of different density classes

Seven Lp(a−) sera exhibited a lipoprotein with pre β_1 mobility upon electrophoresis Six of these were those in which the component had a lower density than 1 050 (and in 4 of which it was clearly demonstrated to be of VLDL nature)

In Table II these 6 samples have been scored as negative for pre β_1 lipoprotein With the exception of 1 sample all the sera containing pre β_1 lipoprotein with a density higher than 1 050 are Lp(a+) In addition 10 samples negative for pre β_1-lipoprotein are Lp(a+)

Table II *Pre β_1-lipoprotein with density above 1 050 g/ml and Lp(a) antigen in 107 50–52 year-old Swedish males*

	Pre β_1 lipoprotein		
	Present	Absent	Total
Lp(a+)	18	10	28
Lp(a−)	1	78	79
Total	19	88	107

χ^2=51 99 1 d.f p<0 0001

Origin

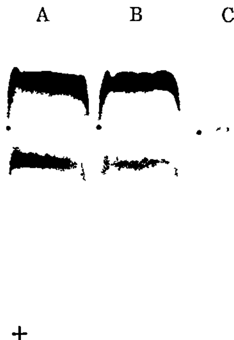


Fig 1 Pre β_1-lipoprotein/Lp(a) lipoprotein (dot) in two normal sera (*A* and *B*) and in the >1 050 density class (*C*) in agarose gel electrophoresis

Serum lipid values in persons positive or negative with respect to Lp(a) lipoprotein/pre β_1-lipoprotein

Table III A and B shows several lipid parameters among positives and negatives for pre β_1 lipoprotein and Lp(a) lipoprotein respectively The pre β_1 lipoprotein positive group had significantly higher amounts of total cholesterol free cholesterol and triglyceride than the negative group and the fraction of esterified cholesterol was higher in the latter A similar tendency towards a difference between the positive and the negative group was likewise found for the Lp(a) antigen with respect to the cholesterol parameters but no suggestion of a difference with respect to triglyceride values None of the differences between the Lp(a+) and the Lp(a−) group were statistically significant

Table III C presents a comparison between the pre β_1 lipoprotein negative group and the 6 samples which had a component with pre β_1 mobility residing in the VLDL fraction of serum (all these 6 sera were Lp(a−)) There were significant differences between the two groups with respect to all 4 lipid parameters

Table III D compares the pre β_1 lipoprotein negative group and the pre β_1 lipoprotein positive group after exclusion of the 6 sera which contained a lipoprotein component of VLDL nature migrating in the pre β_1 region There was no suggestion of a difference between the two groups with respect to triglyceride values whereas the remaining parameters exhibited the same tendency towards differences as those observed in Table III B However only the difference with respect to free cholesterol was statistically significant

DISCUSSION

The result of the association test (Table I) confirmed the previously reported close relationship between Lp(a) antigen and the pre β_1-lipoprotein

It is well documented that the Lp(a) lipoprotein resides in the density class 1 050–1 080 g/ml and the observation by Rider et al (16) that their dominantly inherited sinking pre β lipoprotein is identical with the Lp(a) lipoprotein has been confirmed (5 15) The present ultracentrifuge studies on pre β_1 lipoprotein show that in most cases the lipoprotein fraction with pre β_1 mobility resides in the density class >1 050 g/ml This finding further confirms the close relationship between Lp(a) lipoprotein and pre β_1 lipoprotein The fact that lipoproteins of VLDL nature appeared as pre β_1-components in 6 sera (transitory in 2 of them) shows that lipoprotein electrophoresis alone does not invariably demonstrate the presence of the pre β_1 lipoprotein of density >1 050 g/ml and should be supplemented with flotation characteristics from ultracentrifuge experiments

When the 6 sera containing pre β_1-components of VLDL nature but no pre β_1 fraction of density >1 050 were considered as negative for pre β_1 lipoprotein all but one pre β_1 lipoprotein positive sera were Lp(a+) (Table II) The nature of the component with pre β_1 mobility which resided in the density class >1 050 g/ml in the one exceptional Lp(a−) serum is not yet known When this single serum is disregarded the data in Table II are compatible with the interpretation that the pre β_1 lipoprotein is identical with the Lp(a)

Table III *Cholesterol and triglyceride analyses in 107 presumably healthy 50-52 year old Swedish males*

A Cholesterol and triglyceride values in 107 males with (pre β_1+) or without (pre β_1−) pre β_1 lipoprotein in their sera (mean ±S D)

	Pre β_1+ (n=25)	Pre β_1− (n=82)	t test of difference between groups t	p
Total serum cholesterol (mg/100 ml)	270 3±36 0	251 1±39 1	2 18	<0 05
Free serum cholesterol (mg/100 ml)	90 7±24 1	76 2±17 7	3 28	<0 005
Esterified cholesterol (%)	66 6± 6 8	69 7± 5 1	2 38	<0 02
Triglycerides (mmol/l)	2 4± 1 6	1 7± 0 8	2 85	<0 01

B Cholesterol and triglyceride values in 107 males positive (Lp(a+)) or negative (Lp(a−)) with respect to Lp(a) antigen (mean ±S D)

	Lp(a+) (n=28)	Lp(a−) (n=79)	t test of difference between groups t	p
Total serum cholesterol (mg/100 ml)	259 8±40 4	254 1±38 8	0 65	N S
Free serum cholesterol (mg/100 ml)	84 6±22 3	77 8±19.3	1 53	N S
Esterified cholesterol (%)	67 3± 7 7	69.5± 4 7	1 81	N S
Triglycerides (mmol/l)	1 8± 0 8	1 9± 1 2	0 57	N S

C Cholesterol and triglyceride values in 6 Lp(a−) males with a pre β_1 fraction of VLDL nature (pre β_1 VLDL/Lp(a−)) and in 82 males negative with respect to pre β_1 lipoprotein (pre β_1−) (mean ±S D)

	Pre β_1 VLDL/ Lp(a−) (n=6)	Pre β_1−(n=82)	t test of difference between groups t	p
serum cholesterol (mg/100 ml)	292 3±21 5	251 1±39 1	2 54	<0 02
serum cholesterol (mg/100 ml)	102 3±24 0	76 2±17 7	3 41	<0 001
ed cholesterol (%)	65 3± 5 9	69 7± 5 1	2 01	<0 05
endes (mmol/l)	4 1± 2 4	1 7± 0 8	5 70	<0 001

D Same as A after exclusion of 6 Lp(a−) males with a pre β_1-fraction of VLDL nature (mean ±S D)

	Pre β_1+ (n=19)	Pre β_1− (n=82)	t test of difference between groups t	p
Total serum cholesterol (mg/100 ml)	263 3±37 2	251 1±39 1	1 24	N S
Free serum cholesterol (mg/100 ml)	87 1±23 5	76 2±17 7	2 25	<0 05
Esterified cholesterol (%)	67 1± 7 1	69 7± 5 1	1 83	N S
Triglycerides (mmol/l)	1 9± 0 9	1 7± 0 8	0 77	N S

N.S =not significant

and that the immunological test is more sensitive than electrophoresis in revealing the presence of this lipoprotein

The previously reported strong association (but not identity) between Lp(a) lipoprotein and pre β_1 lipoprotein when whole sera are studied is obviously caused by the close relationship (and probable identity) between pre β_1 lipoprotein with density >1 050 g/ml and Lp(a) lipoprotein No relationship seems to exist between Lp(a) antigen and VLDL components with pre β_1 mobility

Exclusion of the 6 sera containing VLDL com

onents with pre β_1 mobility eliminated the ap arent discrepancy between Lp(a) lipoprotein and re β_1 lipoprotein with respect to lipid values The revious impression that triglyceride levels are gher in pre β_1 lipoprotein positive than in nega ve persons was obviously caused by triglyceride ch VLDL components with pre β_1 mobility sing as true pre β_1 lipoprotein Clearly these LDL components differ from pre β_1 lipoprotein ith a higher density than 1 050 g/ml, not only with spect to density characteristics but also immu ologically The VLDL components with pre β_1 obility do not appear to occur more frequently patients with CHD than in healthy people

The trend (not significant in this study) towards slightly higher serum cholesterol level in posi ves with respect to Lp(a) lipoprotein/pre β_1 lipo rotein is of interest since it tends to confirm our arlier finding (13) However the difference is too mall to support the belief of Walton et al (17) hat the higher frequency of Lp(a+) in patients ith CHD than in healthy people is secondary to igher lipid and LDL levels Furthermore our revious and present data show that we find no ssociation between the phenotype Lp(a+) and tri lyceride level This observation is at variance ith that of Walton et al

The findings by Walton et al of a higher fre uency of Lp(a+) subjects and a higher amount f Lp(a) lipoprotein in selected patients with hyper pidemia than in healthy persons are interesting s much evidence suggests that a high frequency f early atherosclerosis is found among individuals ith high lipid levels These findings are in agree- nent with our data (4 13) The data of Walton et al nd our previous and present findings seem to sup ort our hypothesis that the Lp(a) lipoprotein per se nay be of importance for the early development of therosclerosis

ACKNOWLEDGEMENT

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Predictable Reduction in Anticoagulant Activity of Warfarin by Small Amounts of Vitamin K

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ABSTRACT After withdrawal of warfarin (Marevan), 48–72 hours are required to raise the Thrombotest values from a therapeutic level (range 5–10%) to 12% or higher By i v administration of 1 mg vitamin K1 (Konakion®) and without changing the dose of the anticoagulant, this effect could be obtained within 24 hours Furthermore the effect of vitamin K vanished within 2–5 days The use of small amounts of vitamin K may therefore be a simple and reliable way of obtaining a rapid, temporary reduction in anticoagulant effect

Anticoagulant therapy with vitamin K antagonists is widely used to prevent thrombosis Episodes of spontaneous bleeding are therefore not uncommon Severe bleeding is treated with large doses of vitamin K and transfusions of blood or plasma During minor bleeding or when surgical intervention is necessary only a moderate reduction in anticoagulant effect is desirable in order to reduce the risk of rethrombosis (2 3) Generally this is achieved by partial or total withdrawal of the anticoagulant drug (1)

From the study of van der Meer et al (6) it emerges that i v administration of small amounts of vitamin K1 without changing the dose of anticoagulant might afford a simple and reliable alternative The data presented below are in accordance with this concept

MATERIAL AND METHODS

Twenty five patients who had been treated with warfarin (Marevan) for various periods (2 weeks to 3 years) were studied They were all in a steady state with respect to hypocoagulability their Thrombotest (TT) values being within the therapeutic range (5–10% of normal) Twenty of the patients received 1 mg vitamin K1 (Konakion®) i v without altering the dose of warfarin In the remaining 5 patients anticoagulant treatment was withdrawn Thereafter all patients were followed by repeated TT readings during 48 hours

Reagents

Vitamin K1 Konakion® (Hoffmann La Roche Basle Switzerland) *Sodium warfarin* Marevan (Nyegaard & Co A/S Oslo Norway) *Thrombotest* (Nyegaard & Co A/S Oslo Norway) was carried out according to the manufacturer

RESULTS

In all patients receiving 1 mg vitamin K1 this amount proved sufficient to raise the TT level above 12% within 24 hours (Fig 1 above) In contrast total withdrawal of warfarin did not influence TT appreciably during this period (Fig 1 below)

The effect of vitamin K1 did not seem to depend on the dose of warfarin used to achieve adequate anticoagulant effect (Table I)

In 4 of the 5 patients tested the effect of vitamin K1 vanished within 2–5 days (Fig 2)

DISCUSSION

With the TT method for the control of anticoagulant therapy with vitamin K antagonists values of 5–10% of normal are recommended as optimal (4 5) whereas values above 12% are considered sufficient to prevent excessive bleeding following surgery (7) In the present study the effect of 1 mg vitamin K1 (and sustained dose of anticoagulant) varied considerably from patient to patient but invariably a TT value of at least 12% was achieved within 24 hours the highest value being 32% (Table I) The effect of vitamin K1 disappeared within 2–5

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Normal Iron Absorption Determined by Means of Whole Body Counting and Red Cell Incorporation of ^{59}Fe

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BSTRACT Gastrointestinal iron absorpt on has een measured in 27 apparently healthy subjects (8 nales and 19 females) by means of whole body count ng Whole body retention 14 days after oral dministration of 10 μCi ^{59}Fe with a carrier dose of 9 9 mg Fe^{2} was used as an expression of absorption The percentage incorporat on of the orally applied ^{59}Fe into the total erythrocyte mass within 14 days erythrocyte incorporation) was estimated as well Geometric mean iron absorption was 9 1+2 2 (S D) % in males and 8 3±2 1% in females in the entire series it was 8.5+2 1% The dif ference between males and females was not sig nificant Arithmetic mean red cell utilization of absorbed ^{59}Fe was 92 9±4 0 (S E M) % in the entire series The correlation between iron absorption and erythrocyte incorporation was found to be h ghly sign f cant (r 0 96 $p<0\ 001$)

Among the methods used for determin ng iron absorpt on the one involv ng the appl cat on of ron isotopes and the determ nat on of the r retent on us ng scann ng by whole body counting has been found to be sens tive reproduc ble and easy to handle in the cl n cal rout ne The normal values obtained by the chem cal balance and tolerance tests h therto used wh ch by now are of merely h storical nterest deviate greatly from those obta ned with rad oisotopes At the same t me the absorpt on values observed n stud es us ng whole body count ng are rather confl ct ng partly owing to d fferences in test doses partly to the wide scat ter of b ological data even the populat ons tested may have differed and a vary ng magn tude of the ron depots in populat on samples may have led to differences in the capac ty of the intestinal epi thel um to absorb (3)

The object of the present paper s to subm t th results from a study of ron absorpt on obta ned b whole body count ng in a series of hea thy subj ct In addit on the ncorporat on of the adm n e ed ^{59}Fe into the erythrocytes has been e t m d

CASE MATERIAL

E ght males and 19 females we e e am ne Symptoms of anem a and e od s of hemorrhage had not previously be n r c e the probands they had not been trea d w o and the women had not been p egn n w n months None of the probands h d rved donors Eleven of the subjects we e med a stu d members of the hosp tal staff 16 we e pa ents u from m nor d sorders not nfluencing the on ab o p Hb levels mean corpuscular volume (MCV) and mean corpuscular Hb concentra on (MCHC) were all w h n normal l m ts Intake of drugs was d scon nued at eas 14 days prior to the trial

METHODS

A test solution composed of 10 μC ^{59}Fe n 0 1 mg fer ric trate ferrosulphate (9 9 mg Fe^{2}) and ascorb c acid (10 mg) was prepared The solution was swilled down w h 200 ml drink ng water after an overnight fast and thereaf ter the subjects cont nued to fast for a further two hours Background activ ty was measured before the test solution was given values obtained by counting four hours later were used as 100% value (15) Counting was repeated 12 and 14 days la er in order to check the stabil ty of the values the 14 h-day value i e that obtained after correc t on for background activity and sotope decay was used as an express on of the absorp on of ron

An unsh elded model was used for whole body count ng two 3 nch sod um od de Tl scintillation crystals bu lt nto lead coll mators one below the other above the

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Immunodeficiency and Prognosis in Hodgkin's Disease

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ABSTRACT Blood lymphocytes from the majority of 33 unselected and untreated patients with Hodgkin's disease were deficient in T lymphocytes and their DNA synthesis induced by concanavallin A mitogen and PPD antigen was impaired The spontaneous DNA synthesis during the first 24 hours of culture was often raised The prognostic role of the lymphocyte deficiency was evaluated in a follow up 10–22 months after institution of therapy The lymphocyte functions were more commonly abnormal in patients responding poorly to treatment (in complete remission, relapse after treatment, or death) than in patients entering complete remission The lymphocyte deficiency seems to give information about prognosis in Hodgkin s disease in addition to histopathology, clinical stage B symptoms and age

A more refined diagnostic procedure and a gradual intensification of the therapy have both contributed to a better prognosis for the patient with Hodgkin s disease during the recent 15 years (9 10 14) The change from unmaintained single agent chemotherapy to intensive multiple agent therapy followed by long term maintenance has increased the frequency of remissions and prolonged their duration However treatment is still toxic with acute and late complications The preferred treatment varies mainly with stage and thus indirectly with prognosis Knowledge of the important prognostic factors is essential since an intensive therapy is vital for the risk patient while another patient might be saved from overtreatment Age clinical stage B symptoms and histopathology are well documented prognostic factors in Hodgkin s disease (13 17) Moreover lymphocytopenia has been recognized for many years as a bad prognostic factor at least in advanced disease (3 21)

Generally untreated Hodgkin patients have impaired cell mediated immunity such as impaired delayed hypersensitivity (1) and ability to reject homografts (15) and they are susceptible to various infectious agents (7) Furthermore the patients with Hodgkin s disease usually have low T lymphocyte levels in their blood (4 20) and the lymphocytes are poorly stimulated in vitro by mitogens and by allogeneic lymphocytes to blast transformation and cell division (2 11 16 21) It may be concluded that the immunological abnormalities demonstrated in patients with Hodgkin s disease reflect a T lymphocyte deficiency This impairment of T lymphocyte functions is generally more pronounced in advanced disease (11)

The purpose of this study is to find out whether lymphocyte reactivity is a prognostic factor in addition to age histopathology and stage

MATERIAL AND METHODS

Patients

Thirty three consecutive and previously untreated patients with Hodgkin s disease admitted to Radiumhemmet Karolinska Hospital Stockholm during 1973 were studied There were 21 male patients and 12 female with a mean age of 44 years (range 15–81) All patients were tested before institution of therapy

Staging

Clinical staging was determined from a complete history physical examination biopsies and X ray studies (11) The staging nomenclature according to Ann Arbor recommendation (6) was used

Histopathology

All 33 patients had Hodgkin s disease confirmed by biopsy in the Department of Pathology Radiumhemmet The Rye modification nomenclature (18) was used LP=lymphocyte predominance NS=nodular sclerosis MC=mixed cellularity LD=lymphocyte depletion

Treatment

After staging and immunologic evaluation LP and NS patients stage I and II with a favourable setting that is except left sided supradiaphragmal lymph node engagement received extended field irradiation over the involved half of the body All stage IV patients were treated with a modified MOPP therapy that is cyclophosphamide vinblastine procarbazine hydrochloride and prednisolone for ten days repeated every month Remaining patients were given total nodal irradiation

Evaluation of therapy

The response to therapy was evaluated 10–22 months after institution of therapy The patients were divided into two clinical groups one with patients in complete remission and the other with patients who were in incomplete remission and who had relapsed or died in Hodgkin s disease A complete remission was defined as disappearance of all masses and enlarged nodes and all signs of active disease including hepatomegaly splenomegaly bone marrow infiltration pruritus night sweats fever and increased ESR

Controls

Twenty three healthy persons were used as controls (mean age 35 years range 20–64) All had normal blood lymphocyte counts One healthy control was included in each lymphocyte experiment

Lymphocyte preparation

Lymphocytes were obtained from defibrinated venous blood by sedimentation in gelatine (8) The cells were washed in Hanks solution with 0 5 M Tris buffer pH 7 4 (HT) Phagocytic cells were removed after ingestion of iron powder by a magnet (19) Lymphocytes were then taken off for DNA synthesis experiments while lymphocytes to be tested with cell surface markers were further centrifuged through a Ficoll Isopaque gradient in order to remove remaining red blood cells (5)

ay for lymphocytes binding sheep d blood cells (T lymphocytes)

ymphocyte suspension was mixed with an equal volume of 0 5% washed sheep red blood cells (SRBC) and incubated for 15 min at 37°C The mixture was centrifuged at 90 *g* for 6 min and incubated on ice over night The cells were carefully transferred with a Pasteur pipette to a glass slide and covered with a cover slip Two hundred lymphocytes were counted and cells forming rosettes with ≥3 SRBC were considered positive (E binding lymphocytes) (12)

Assay for immunoglobulin (Ig) bearing lymphocytes

Lymphocytes were incubated with polyvalent rabbit anti human Ig serum (Cappel Lab Inc Downingtown USA) for 45 min at 4°C After incubation sheep anti rabbit Ig serum conjugated with fluorescein isothiocyanate (Statens Bakteriologiska Laboratorium Stockholm Sweden) was added and incubated for 45 min at 4 C After washing the cells were mounted on a glass slide in one drop of 50% glycerol in phosphate buffer pH 7 8 with NaN_3 (1 mg/ml) Membrane fluorescence was examined in a Zeiss fluorescence microscope and 200 cells were counted (12)

EAC binding lymphocytes

A 5% SRBC (E) suspension was mixed with an equal volume of rabbit anti SRBC serum diluted 1 2000 and incubated for 60 min at 37°C The cells were washed twice and adjusted to a 5% suspension (EA-cells) Fresh human AB serum from the same donor as a source of complement was added in dilution 1 20 After incubation at 37°C for a further 60 min the cell suspension was washed twice and adjusted to a 0 5% suspension (EAC) 0 25 ml lymphocyte suspension (10^6 cells) was mixed with 0 25 ml of EAC suspension After centrifugation and incubation at 37°C for 15 min most of the supernatant was discarded and the cells were vigorously resuspended on a whirl mixer The percentage of the EAC binding lymphocytes attaching three or more SRBC was determined by counting 200 cells Lymphocytes incubated with SRBC with rabbit antibody only were included in each experiment (12)

DNA synthesis in lymphocytes

Iron purified lymphocytes suspended in culture medium (RPMI 1640 with 10 mM Hepes, 2 mM glutamine 100 IU penicillin and 100 μg streptomycin/ml) supplemented with 15% heat inactivated human AB serum were added to sterile conical glass tubes and mitogens and antigens were added (see below) Lymphocytes were incubated for 24 and 68 hours respectively Thymidine 2 ^{14}C (0 1 μCi/tube specific activity 10 or 60 mCi/mM) was added at time zero or after 44 hours Incubation was stopped after 24 hours with radioactive thymidine by cooling and the radioactive DNA protein was precipitated by a trichloroacetic acid procedure The radioactivity was measured in a Packard Liquid Scintillation Counter (11) The results given represent mean values of duplicate tubes Since ^{14}C thymidine of two specific activities was used a stimulation index was calculated to describe the DNA synthesis in patients and controls

$$\text{stimulation index} = \frac{\text{experimental c p m}}{\text{median c p m in controls}}$$

The means and the statistical variation of the indices in the two control materials were the same as revealed by the *F* test Therefore the materials were pooled

Mitogen and antigen

Concanavallin A (ConA Pharmacia Fine Chemicals AB Uppsala Sweden batch No 4000) was used in concentrations of 5–80 μg/ml However due to lack of lymphocytes DNA synthesis could not be tested in some preparations at all ConA concentrations Purified protein derivate of tuberculin (PPD Statens Bakteriologiska Laboratorium Stockholm Sweden batch Nos RT27 and RT32 which in pilot experiments stimulated equally well) was used at concentrations of 2 5 and 25 μg/ml

Lymphocyte scoring system

For evaluation of the prognostic significance of lymphocyte functional tests a scoring system was designed based on four lymphocyte parameters lower than range of control group 1) the level of total E binding lymphocytes 2) the DNA synthesis induced by a mitogen ConA 3) the DNA synthesis induced by an antigen PPD and higher than range of controls 4) the spontaneous lymphocyte

Table I *Response to therapy in relation to age and lymphocyte score*

	Complete remission	Incomplete remission *relapse or* death
Total	18	15 (3 dead)
Clinical stage I–II	10	3
Histopathology NS+LP	12	3
Mean age±S E	32 9±3 6	56 3±5 2 (p<0 001)
Mean lymphocyte score±S E	1 2±0 2	2 6±0 3 (p<0 001)

DNA synthesis during the first 24 hours Each abnormal test scores 1

Combined prognostic score

An extended prognostic score system was designed where the summarized significance of lymphocyte score age score (≤40 years 1 40–60 years 2 ≤60 years 3) stage score (I 1 II 2 III 3 IV 4) symptom score (A 0 B 2) and histopathological score (LP 1 NS 2 MC 3 LD 4) was calculated

Statistical analysis

Conventional methods have been used for the calculation of the arithmetic mean and S E M The significance of differences of means was tested by Student s *t* test The limit of statistical significance was considered to be a *p value of 0 05*

RESULTS

Ten to 22 months after instituted therapy 18 patients had responded well and were in complete remission while 12 patients were non responders partial responders or had relapsed (Table I) Three patients had died As expected there is a predominance of clinical stage I–II cases in the complete remission group ten patients compared to three in the incomplete remission group Twelve of 18 patients were classified as nodular sclerosis and lymphocyte predominance in the complete remission group while only three patients with this histopathologic picture were seen in the other group The mean age as well as the lymphocyte score were significantly lower (p<0 001) in the complete remission group There was a tendency to higher lymphocyte score in stage III–IV patients than in stage I–II patients in the complete and incomplete remission groups respectively This increase was not statistically significant (Table II)

In order to study the lymphocyte activity of a rather homogeneous group of patients according to stage and histopathology twenty patients all stage III–IV A and B were compared with regard to their *response to therapy Eight patients were in com*plete remission while 12 belonged to the group of partial or non responders (Table III) The mean age was lower in the former group although not statistically decreased compared to the other group There was a tendency to lower total lymphocyte and T lymphocyte counts in the incomplete remission group Moreover the response to stimulation with ConA and PPD was decreased in this patient group The spontaneous lymphocyte activity was increased in the incomplete remission group None of these differences were statistically significant partly due to the small number of patients in each group On the other hand when the results were summarized in the form of a lymphocyte score there was a significantly higher score in the incomplete remission group Among all stage III–IV A and B patients there is a significant negative correlation between age and response to ConA stimulation (r=−0 65 p<0 01) No correlation with age was noted for lymphocyte counts T lymphocyte counts response to PPD stimulation or spontaneous DNA synthesis

No differences in the number of Ig positive or EAC binding lymphocytes have been noted with regard to age histopathology clinical stage lymphocyte score or response to therapy

In order to study the combined prognostic significance of lymphocyte score age clinical stage B symptoms and histopathologic picture a multivariant score was presented (Table IV) When all patients were compared according to response to therapy there was a highly significant increase in total score in the incomplete remission group which was valid for all single prognostic parameters except for the staging score The highest

Table II *Lymphocyte score (mean±S E) correlated to stage and response to therapy*

Stage	Complete remission	Incomplete remission relapse or death	p
I–II A and B	0 8±0 3 (n=10)	2 0 (n=3)	
III–IV A and B	1 8±0 4 (n=8)	2 8±0 3 (n=12)	<0 05

Table III *Lymphocyte activity in HD patients stages III–IV A and B (mean ± S E)*

	Complete remission (n=8)	Incomplete remission relapse or death (n=12)	p
Age (y)	*40 4 ±6 7*	*55 5 ±5 8*	n s
Total lymphocytes (mean log no /mm³)	3 14±0 05	3 01±0 06	n s
T-lymphocytes (mean log no /mm³)	2 94±0 06	2 85±0 05	n s
DNA synthesis (stimulation index)			
Induced by $ConA_{20}$	0 65±0 18	0 26±0 10	n s
Induced by PPD_{25}	0 60±0 27	0 46±0 20	n s
Spontaneous	4 97±2.5	8 46±2 1	n s
Lymphocyte score	1 8 ±0 4	2 8 ±0 3	<0 05
Total score	9 6 ±0 5	12 7 ±0 8	<0 01

n s =not significant

t value was found for the lymphocyte score, followed by the histopathologic score If the lymphocyte score is excluded from the total score, the difference in total score would still be statistically significant (p<0 001) but with a lower t value (4 77–4 07)

DISCUSSION

studies concerning the immunologic status of with Hodgkin s disease have been pre in recent years most of them reporting vari us defects in immunological functions In this study 33 untreated consecutive patients with Hodgkin s disease have been examined regarding the function of lymphocytes in peripheral blood Generally, these patients had lower numbers of lymphocytes and T lymphocytes in their blood compared to healthy controls (11) No significant alteration was noticed in the number of B lymphocytes (Ig positive and EAC binding lymphocytes) Moreover the response to mitogens (ConA phytohaemagglutinin and pokeweed mitogen) and one antigen (PPD) *in vitro* was markedly decreased compared to healthy controls (11) Immunologic defects were found in 29 of 33 patients with a tendency to more severe impairment in patients with advanced disease

This study has focused attention on the possible correlation between lymphocyte activity and prognosis The patients have been divided into two groups according to response to therapy, one group consisting of patients in complete remission and the other of the remaining patients In all patients there was a good correlation between the responses to ConA and the two other mitogens When the two groups of patients were compared the greatest difference was found for ConA induced stimulation The number of total T-lymphocytes the response to ConA and to PPD stimulation and the spontaneous lymphocyte DNA synthesis were chosen to create a lymphocyte scoring system in order to simplify the evaluation of results It is generally believed that the first three parameters reflect different T lymphocyte functions while the lymphoid cells responsible for the spontaneous lymphocyte activity have not been identified

The results presented above clearly show the differences in lymphocyte score in patients of various clinical stages and histopathology and with varying response to therapy However the lymphocyte reactivity of five patients under 50 years with advanced disease but without B symptoms who

Table IV *Combined prognostic score (mean ± S E) in Hodgkin s disease*

Range within parentheses

Score	Complete remission (n=18)	Incomplete remission relapse or death (n=15)	t value	p value
Lymphocyte	1 22±0 24 (0–3)	2 60±0 26 (1–4)	3 96	<0 001
Age	1 33±0 17 (1–3)	2 20±0 25 (1–3)	3 06	<0 005
Staging	2 39±0 19 (1–4)	2 93±0 21 (2–4)	1 98	n s
Symptom	0 22±0 16 (0–2)	0 93±0 27 (0–2)	2 41	<0 025
Histopathologic	2 11±0 18 (1–3)	3 13±0 25 (1–4)	3 51	<0 005
Total	7 28±0 60 (3–11)	11 80±0 79 (7–16)	4 77	<0 001

were not in complete remission did not differ from that of the remaining 7 patients in incomplete remission This observation suggests that severely impaired lymphocyte activity has an ominous prognostic significance However the number of patients in this report is too small to safely evaluate the significance of each prognostic factor Summarized influence of age symptoms histopathology clinical stage and lymphocyte reactivity on prognosis was studied in a total score resulting in highly significant differences between patients in complete and incomplete remission Further results will be presented after a longer observation time and with a greater number of patients

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Studies on the Haematologic and Cytogenetic Effect of Lithium

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ABSTRACT The corpuscular elements of the peripheral blood and the morphology and chromosome constitution of bone marrow cells have been studied in patients before and during lithium therapy as well as in patients who had received lithium for a substantial period Lithium therapy produced elevated total white cell granulocyte, eosinophil and thrombocyte counts and a lymphocytopenia, whereas no effect was seen on the erythrocyte and reticulocyte counts There were no morphological or cytogenetic changes in the bone marrow aspirates Neither were toxic doses of lithium able to produce chromosome abnormalities in rats

For more than 20 years lithium has been a valuable drug in the treatment of manic depressive illness (21 22 24) At the same time numerous reports dealing with various metabolic and endocrine effects related to lithium therapy have appeared in the literature Thus it has been well documented that lithium may have an antithyroid effect (1) influence carbohydrate metabolism (16 27) and produce a diabetes insipidus like syndrome of renal origin (17 28) Some authors have demonstrated a stimulatory effect of lithium on the adrenocortical activity (15 26) whereas others have not been able to confirm this finding (18) It is well established that lithium may have an effect on the haemopoietic system As early as in 1966 it was noted that an increase in leukocyte count often occurs in patients treated with lithium (12) and recently some evidence has been presented that lithium may have a cytogenetic effect on human peripheral blood lymphocytes (3)

The aim of the present report has been to study the effect of lithium on bone marrow morphology and peripheral blood cell counts in patients who have been treated with the drug for various periods Furthermore we have examined whether lithium has any in vivo effect on the chromosomal constitution of human and rat bone marrow cells Finally the effect of lithium on the granulocyte count was studied in three patients with idiopathic granulocytopenia.

MATERIAL AND METHODS

Four groups of patients were studied Group I consisted of 36 psychiatric patients 20 males 16 females aged 22-68 years who had been treated with lithium in a daily dose of 900-1 500 mg for two months to ten years The lithium level in serum was 0 8-1 2 mmol/l Twenty of the patients received no other therapy whereas 16 also took one of the following drugs doxepine perphenazine procyclidine imipramine amitriptyline chlorprothixene levothyroxine digoxin or a diuretic None of the patients suffered from a haematologic or infectious disease

Group II—the control group—consisted of 36 normal persons 18 of either sex aged 20-70 years None of them received any drugs

Group III consisted of ten patients—five of either sex—aged 20-79 years without any haematologic or infectious disorder Most of the patients were treated with doxepine digoxin diuretics or salicylates However the medication was not changed during the study

Group IV consisted of three female patients aged 19-74 years with idiopathic granulocytopenia

In groups I and II the following values were determined Hb concentration erythrocyte reticulocyte leukocyte granulocyte lymphocyte eosinophil and thrombocyte counts and vitamin B_{12} concentration in serum and the two groups were compared with respect to each of these parameters by a Wilcoxon test In seven of the patients in group I bone marrow aspirates were performed which

Table I *Haematological changes before and during treatment with lithium carbonate in 10 hospitalized patients*

Pat no		Hb (mmol/l)	Erythrocytes $\times 10^{12}$/l	Total leukocytes $\times 10^9$/l	Thrombocytes $\times 10^9$/l	Vitamin B_{12} (pmol/l)	Granulocytes $\times 10^9$/l	Lymphocytes $\times 10^9$/l
1	Predrug	8.5	4 34	7 6	259	438	5 85	1 44
	On drug							
	1 week	8 0	4 17	8.5	325	436	7 06	1 [illegible]
	2 weeks	8.2	4 20	8 0	510	466	6 16	1 69
2	Predrug	8 1	4 27	7 1	222	418	5 04	1 78
	On drug							
	1 week	7 7	4 11	9 2	246	394	7 73	1 29
	2 weeks	7 7	4 05	8 7	286	397	7 48	0 78
3	Predrug	8 3	4 52	4 5	241	486	2 84	1 49
	On drug							
	1 week	8 2	4.58	6 1	273	439	3 90	1 71
	2 weeks	7 9	4 31	5.5	317	385	3 80	1 38
4	Predrug	7 7	4 09	7 3	240	411	3 58	3 43
	On drug							
	1 week	7 9	4 30	11 2	367	617	7 73	3 14
	2 weeks	7 7	4 13	7 8	344	502	5 23	2 18
5	Predrug	7 8	4 11	6 1	178	452	3 36	[illegible] 50
	On drug							
	1 week	8 6	4 57	6 6	208	492	4 69	1 39
	2 weeks	8 8	4 54	7 4	189	440	4 96	2 00
6	Predrug	8 4	4 53	4 3	284	326	2 32	1 89
	On drug							
	1 week	8 6	4 78	5 3	325	369	3 18	2 07
	2 weeks	8 6	4 48	5 9	333	358	4 31	1 48
7	Predrug	8 5	4 64	5 0	255	282	3 30	1 35
	On drug							
	1 week	7 9	4 32	4 8	298	354	3 26	1 34
	2 weeks	8 3	4.53	5.3	268	364	3.55	1 75
8	Predrug	9 0	4 70	5 6	335	601	3 25	2 30
	On drug							
	1 week	9 1	4 87	9 0	356	577	6 66	2.25
	2 weeks	8 7	4 60	7 5	303	532	5.55	1 88
9	Predrug	9 1	4 14	6 3	263	559	2 84	2.58
	On drug							
	1 week	8 4	4 31	7 2	319	455	3 96	2 95
	2 weeks	7 9	3 90	9 7	305	488	6 89	2 81
10	Predrug	7 6	4 18	4 7	271	427	2.59	1 93
	On drug							
	1 week	8 1	4 41	5 8	327	490	3 19	2.49
	2 weeks	7 9	4 30	8 5	340	532	6 72	1.53

cyte count ($0\ 001<p<0\ 01$) the granulocyte count ($p<0\ 001$) the eosinophil count ($0\ 01<p<0\ 05$) and the thrombocyte count ($p<0\ 001$) were significantly higher in group I than in group II The lymphocyte count was significantly lower in the treated patients than in the controls ($p<0\ 001$) There was no difference between the two groups as regards the values for Hb and vitamin B_{12} concentrations the erythrocyte and the reticulocyte counts ($p>0\ 05$)

Table I shows the results of the laboratory studies in ten patients before and after one and two weeks therapy After one week a significant increase was seen in the granulocyte ($p=0\ 05$) and thrombocyte counts ($0\ 01<p<0\ 05$) After two weeks the p values were $<0\ 01$ and $0\ 01<p<0\ 05$ respectively After two weeks there was also a significant increase in the total leukocyte count ($0\ 01<p<0\ 05$) As regards the other laboratory results no change was seen

In three patients with granulocytopenia neither the granulocyte nor the thrombocyte count w

Table II *Chromosomal findings in bone marrow cells from seven lithium treated male patients*

Pat no	Age (y)	Total cells (counted)	Cells with structural abnormalities (%)	Hyperdiploid cells (%)
1	28	50	2	0
2	66	50	0	0
3	26	50	2	0
4	39	50	0	0
5	34	50	0	0
6	56	50	4	0
7	36	50	0	0
Total			1 1	0

raised during therapy with lithium In one of the patients who suffered from cyclic granulocytopenia no change of the cycles occurred during three months therapy with lithium

No abnormalities were found in the smears from the bone marrow aspirates from the seven patients treated with lithium The myeloid erythroid ratio was 2 9 1 No increase was found in the number of megakaryocytes

The results of the chromosome studies in seven patients treated with lithium are given in Table II The number of hyperdiploid cells or metaphases with structural aberrations was not increased The control values of our laboratory are 0 2 and 1 4% spectively

able III lists the results of chromosome studies in treated with lithium These values do not differ our control values either being 0 4% hyperdiploid metaphases and 0 0% metaphases with structural aberrations

DISCUSSION

Several authors have shown that the WBC may be elevated in patients treated with lithium (12 13 14) In the present series the increase in the leukocyte count was moderate Thus the WBC did not exceed 11 000/μl in any of our patients treated with lithium The mean leukocyte count in the treated group was 7 560 as compared to 6 180 in the control group In the group of patients studied before and during therapy there was a significant increase in granulocyte counts but not in total WBC

However other reports noted a considerable *leukocytosis in some patients Thus Shopsin et al* (25) found mean pretreatment values of 9 116/μl (range 4 200–14 000) as compared to treatment values of 14 716/μl *(range 5 900–24 000) The magni*tude of the leukocyte count increase appears to be independent of the dosage or blood concentration of lithium (25)

The present results show that the increase in WBC is due to a considerable granulocytosis without the appearance of immature myeloid cells in the peripheral blood At the same time there is a slight lymphocytopenia

The cause of the granulocytosis in lithium treated patients is not known It has been proposed that the possible stimulatory effect of lithium on the adrenal cortex may be the mechanism underlying the granulocytosis and lymphocytopenia in the peripheral blood (15, 26) Recently however evidence has been presented that the granulocytosis may be due to an effect of lithium on the bone marrow Thus Tisman et al (30) found that lithium was able to stimulate human granulocyte colony growth in vitro The same authors also demonstrated that serum unsaturated B_{12} binding capacity was significantly elevated in patients treated with lithium This finding suggests that there is an absolute increase in the body granulocyte pool (2) In the present series we were not able to determine the vitamin B_{12} binding proteins in serum however we did not find an elevated vitamin B_{12} concentration in the serum of the lithium treated patients The present study lends further support to the concept that lithium exerts an effect on the bone marrow and that the peripheral blood changes are not due to a hypercorticism Thus we found an increased eosinophil count in the treated patients whereas eosinopenia is characteristic of excess production of corticosteroids (19) Furthermore the increased thrombocyte counts *demonstrated in the lithium treated patients of the* present series speak against the theory of hypercorticism as the cause of the peripheral blood changes

Table III *Chromosomal findings in bone marrow cells from lithium treated rats*

Group	Total cells (counted)	Cells with structural abnormalities (%)	Hyperdiploid cells (%)
A	250	0	0
B	250	0	1 2

In this study elevated thrombocyte counts were found both in the chronically treated patients and in those who had received lithium for one and two weeks only This finding has not been described before The fact that the granulocyte and thrombocyte counts are raised in lithium treated patients whereas the erythrocyte and reticulocyte counts are normal suggests that lithium either exerts a stimulatory effect on granulocyte and thrombocyte precursors in the bone marrow or may influence cells in the stem cell compartment to differentiate to precursors of granulopoiesis and thrombopoiesis

It has been proposed that lithium may be of therapeutic value in patients with granulocytopenia (30) However the negative findings in the three granulocytopenic patients in the present report give no support to this assumption

Lithium therapy is often indicated in pregnant women as the manic-depressive illness frequently makes its debut in the reproductive years This fact naturally raises the question whether lithium has teratogenic properties Studies on lithium teratogenesis in mammals are inconclusive An increased incidence of cleft palate has been described in mice but the lithium doses were so high that there was a 37% incidence of maternal death (20) Developmental defects were also demonstrated in rats treated with sublethal dosages (32) Other studies with rats (5 7 8 31) rabbits (5) and monkeys (5) were not able to show any teratogenic action of lithium No evidence has been presented that lithium possesses teratogenic properties in man Of 118 children born by lithium treated women nine were born with abnormalities (23)

As there is some evidence that chromosome breaking agents may also have teratogenic properties cytogenetic studies have recently been applied in teratologic research Both in vivo and in vitro chromosome studies have been performed with lithium In 1969 Friedrich and Nielsen (3) in three lithium treated patients demonstrated an increased number of breaks in metaphases from the peripheral blood lymphocytes In vitro studies performed by the same authors showed an increase in hyperdiploid cells when toxic doses of lithium were added to the culture medium Similar investigations by other authors have not been able to ascertain any cytogenetic effect of lithium (4 6 29)

We studied the chromosome constitution of bone marrow cells from lithium treated patients because the bone marrow findings seem to be more in accordance with the in vivo conditions than the study of the peripheral blood lymphocytes (10) which may be subject to alterations due to in vitro culture We were unable to find any deviation from the normal findings as regards either structural or numerical abnormalities At the same time we found no evidence of a cytogenetic effect of lithium in bone marrow chromosomes of rats receiving toxic doses of lithium the daily dose being about 30 times the common therapeutic dose in man These findings seem to exclude a cytogenetic effect of lithium in man and rats

ACKNOWLEDGEMENT

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The Effect of Glycerol Infusion in Acute Cerebral Infarction

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ABSTRACT Intravenous administration of glycerol has been used in an effort to improve the prognosis for patients with acute cerebral infarction Fifty patients were treated actively and 56 served as a control group The neurological status before, during and after treatment for six days was assessed by a score system The patients were grouped according to the initial score Those with the highest and lowest scores did not improve from glycerol infusion However, the treated patients with intermediate scores, where the prognosis is known to be dubious, showed a significant improvement compared to the controls The mortality was not influenced No undesirable side effects from glycerol were registered

The possibilities of preventing cerebrovascular strokes have increased considerably during the last decade but when a stroke is manifest there is far less chance to influence the prognosis In selected cases acute operation may be carried out aiming for instance at the evacuation of a haematoma For most patients especially those with cerebral infarction this type of treatment must be far from ideal In such cases conservative therapy aiming to minimize the area of irreversible destruction should be preferable In cerebral infarctions it is well known that the ischaemic oedema reaches its peak within the first week after the onset of the stroke (6) Thus it is natural that various methods have been used in efforts to reduce the initial oedema such as treatment with steroids mannitol urea or dextran

Recently the use of i v glycerol infusion has been suggested by Mathew et al (4) This treatment is based upon the principle that glycerol acts as a hyperosmolar agent In addition it improves cerebral metabolism The latter mechanism is supposed to be mediated through an initiation of the recoupling of uncoupled oxidative phosphorylation in the ischaemic brain tissue Glycerol may act as a source of glucose without insulin and thus facilitates the cerebral metabolism as it passes the blood brain barrier Glycerol may also have a direct effect on the membrane function as it has been shown that glycerol participates in active transport across cell membranes in the form of α glycerol phosphate (5 7)

In a previous paper (3) we have used a score system suggested by Mathew et al (4) to predict the short term outcome for cerebrovascular lesions We were able to show that the outcome within one month for cerebrovascular lesions bad or favourable could be predicted very accurately in most cases For an intermediate group with scores from 20 to 40 the outcome was less predictable

We then decided to make an attempt to find out whether the prognosis for cerebral infarctions could be improved by means of i v glycerol infusion The intermediate group was of course of special interest

MATERIAL AND METHODS

A series of 106 consecutive patients below the age of 80 were collected for the trial during a period of 14 months In all cases the diagnosis of cerebral infarction was based on the standard criteria discussed in a previous paper (2) The patients were all first time lesions and admitted to the hospital within 24 hours after the ictus No patients were accepted for the study later than 24 hours from the onset of symptoms Patients born on even dates were chosen for active treatment with glycerol those born on uneven dates served as controls

All patients went through a general medical examination and neurological evaluation by one of the authors The neurological deficit was assessed by following the score system of Mathew et al (4) which has been slightly modified by us (3) (Table I) In all cases a lumbar puncture echoencephalography and scull X-ray were performed

Table I *Neurological evaluation of patients with acute stroke*

Factor	Score
Mentation	
Level of consciousness	
Fully conscious	8
Somnolent	6
Precomatose	4
Comatose	0
Orientation	
Orientated×3	6
Orientated×2	4
Orientated×1	2
Disorientated	0
Speech	
Normal	23
Disconnected phrases	15
Expressive or impressive aphasia	10
Dumb	0
Cranial nerves	
No conjugate deviation	6
Conjugate deviation	0
Central facial function	
Intact	3
Palsy	0
Motor strength (each limb separately)	
Normal	5
Paresis	2
Paralysis	0
Performance disability status scale	
Normal	28
Moderate impairment	21
Considerable impairment	14
[illegible] impairment	7
[illegible] performance at all	0
Reflexes	
Normal	3
Spastic	1
No reflexes	0
Sensation	
Normal	3
Mild sensory abnormality	2
Severe sensory abnormality	1
No response to pain	0
	100

acutely Red and white blood cells were counted routinely ESR creatinine cholesterol triglycerides urea and electrolytes were determined Patients with signs of cerebral haemorrhage or embolism were excluded as were patients with systematic disease such as SLE or leukaemia cardiac or renal failure In the group with active treatment i v glycerol administration was started within 24 hours Glycerol was administered in the form of 50 g glycerol mixed in 500 ml 5% glucose in 25% physiological saline The infusions were administered during 6 hours and were repeated daily for 6 days

All patients were examined daily by one of the authors and an assessment was according to the score system No other hyperosmolar active agents were used nor steroids The nursing and physiotherapy were the same for comparable cases in both groups

For calculation of statistical differences the ordinary χ^2 test with Yale s correction was used

RESULTS

Fifty patients aged 58–79 years (mean 68 3) were treated Fifty six patients aged 56–79 years (mean 70 7) *were placed in the control group Thirty seven* patients died 14 in the group with treatment and 23 in the group without As the immediate prognosis varies greatly with the scores (3) the patients were grouped according to the score shown in Table II Table III shows the mean score for the surviving patients on each day, together with the statistical difference between the gain in score during the observation time for the two groups It will be seen that there is no difference between the treated and untreated groups for scores under 20 and over 40 However for the intermediate group, scores 20–39 the difference between the groups is highly significant ($p<0\ 01$) in favour of the treated group The improvement seems to be most pronounced during the latter part of the observation time

A closer analysis shows that the gain in scores is mostly due to regaining consciousness and speech There is no difference in the mortality for treated and untreated patients in any of the groups

DISCUSSION

One of the difficulties encountered in this type of study is to evaluate the comparability of treated and untreated patient groups This is especially true

Table II *Distribution of the patients within the score groups*

	Treated		Untreated		
Score	Men	Women	Men	Women	Total
0–19	6	4	9	9	28
20–39	10	18	10	14	52
40–	6	6	6	8	26
Total	22	28	25	31	106

Table III *Mean score for surviving patients on each day of treatment*

Score group		Day of treatment 1	2	3	4	5	6	No of deaths
0–19	Treated	12 1	13 1	10 2	10 2	8 2	9 1	8
	Untreated	13 1	10 1	9 2	11 1	12 1	10 3	16
		χ^2=1 415 n s						
20–39	Treated	22 1	23 1	25 5	26 8	30 2	37 2	6
	Untreated	25 1	23 0	24 1	24 9	27 8	25 6	7
		χ^2=16 221 p<0 01						
40–	Treated	60 0	65 3	69 0	71 0	73 2	74 3	0
	Untreated	55 8	57 6	58 2	60 8	67 2	65 1	0
		χ^2=6 897 n s						

when dealing with patients with neurological disability

The use of a score system and subdivision of patients according to the scores offers advantages and reduces the influence of differences in spontaneous regressions not referable to e g sex and age differences but attributable to the extent of the symptomatology To our knowledge this technique has not been used previously in attempts to evaluate therapeutic methods in cerebrovascular strokes

Our results confirm the earlier observation that i v administered glycerol may be of benefit in patients suffering from acute cerebral infarction However this seems to be true only for certain types of patients The ominous prognosis of a very low score which has been demonstrated earlier (3) does not seem to be influenced at all The prognosis for patients with scores over 40 is so good per se that the glycerol infusion makes no difference

The beneficial effect of the glycerol treatment is entirely registered in the intermediate score group This difference is most probably real as the treated and untreated groups were very homogeneous as regards age sex and initial degree of disability

The overall mortality was found to be significantly higher in the untreated group This difference however should probably not be ascribed to the treatment but rather to the fact that the control group comprises a higher number of patients with a very low score e g with almost 100% mortality There were no differences between treated and untreated patients within corresponding score groups

The score difference of 10–15 points may not look impressive in itself But it should be born in mind that this means for example the difference between unconsciousness and consciousness regaining speech facilities or the ability to swallow or the practical use of an arm and a hand It is thus apparent that the degree of improvement will be of considerable immediate importance for nursing and rehabilitation The question then arises Will this glycerol treatment also affect the long term prognosis? The limited number of patients included in this study and the short time of follow up does not permit any conclusions To judge from our previous experience (1 2 3) two reasonable assumptions may be allowed Firstly a substantial number of patients in the intermediate group have presented a score improvement which at this score level must mean a considerably diminished handicap Secondly the improvement may also influence the life expectancy especially for younger individuals

The use of glycerol offers certain advantages It is easy to administer and avoids the disadvantages of steroids which are sometimes used for the same purpose No side-effects were observed and in particular there was no rebound phenomenon

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Factors Related to Blood Pressure in a General Population Sample of Swedish Men

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ABSTRACT Blood pressure (BP), heart rate (HR), height, weight, hereditary factors, smoking habits and physical activity have been determined in a random population sample of men aged 47–54 years attending a screening examination, which was part of a multifactor primary preventive trial Of the 9 967 men who were invited to the study, 83 % answered a postal questionnaire and 75 % took part in the screening examination Of all persons not on antihypertensive treatment, 10 % had systolic blood pressure (SBP) >175 mmHg and 6 % had diastolic blood pressure (DBP) >115 mmHg Of those on hypotensive treatment 43 % had SBP >175 mmHg and 30 % had DBP >115 mmHg indicating that the hypertension was not well controlled A weak positive correlation was found between HR and BP and between relative body weight (RBW) (defined as [weight/height] – 100) and BP Both HR and RBW were higher in the treated hypertensives than in the remainder of the population studied Subjects with a positive heredity for myocardial infarction or stroke had a significantly higher BP than persons with a negative heredity, but the absolute differences were small A weak relationship was demonstrated between smoking habits and DBP, with the lowest BP in persons smoking >25 cigarettes a day There was no relationship between the degree of physical activity and BP

Previous epidemiological studies have analysed the relationships between blood pressure (BP) and heart rate (HR) (11) relative body weight (RBW) (3 13 25 26) heredity for cardiovascular and cerebrovascular diseases (1 8 19 25) smoking habits (13 26) and physical activity (5 16 17) The results have varied somewhat probably due to differences in the selection of material

In 1970 a multifactor primary preventive trial against cardiovascular diseases was started in Goteborg (31) The initial screening examination was completed in 1973

The aim of the present communication was to 1 Describe the population with regard to BP HR and RBW 2 Describe the relationships between BP and age height weight RBW and HR 3 Answer the following questions (*a*) Do subjects on antihypertensive treatment differ from those without such treatment with regard to BP HR and RBW? (*b*) Do subjects with heredity for myocardial infarction and cerebrovascular disease have a higher BP than subjects without such heredity? (*c*) Are there differences in BP between groups with different smoking habits or different physical activity?

MATERIAL

The present material was recruited from a screening examination which was a part of a primary preventive trial (31) The study group consisted of all men born in 1915–22 and 1924–25 and living in Goteborg Sweden (n=9 967) The participants were 47–54 years old at the time of the screening examination Of the 9 967 men 8 223 (83 %) answered an illustrated postal questionnaire and 7 452 (75 %) took part in the screening examination Of these 7 452 subjects 420 (5 6 %) were on antihypertensive treatment In 189 subjects (2 5 %) it could not be decided whether they were on antihypertensive treatment or not These subjects were excluded from further analysis

Of the 6 843 subjects not on antihypertensive treatment BP was recorded in 6 794 HR in 6 773 and RBW in 6 786 Of the 420 subjects on antihypertensive treatment BP was recorded in 417 HR in 414 and RBW in 418

The non participation rate was 25% General characteristics of this group have been analysed (30) A small proportion of the non participants among those born in 1921 did take part in an examination after special reminders (n=38) No differences in BP HR RBW heredity

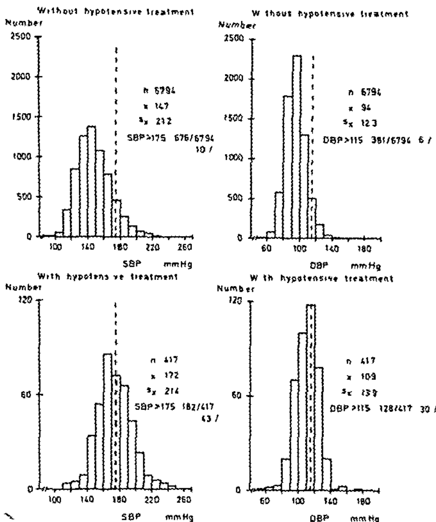


Fig 1 Systolic (*SBP*) and diastolic (*DBP*) blood pressure in subjects with and without hypotensive treatment at the time of the screening examination

for cardiovascular and cerebrovascular diseases smoking habits or physical activity were found between those taking part in the initial screening examination and those taking part after reminders

METHODS

The screening procedure has been described in detail earlier (29 31) The screening examination started in 1970 with those born in 1915 and ended in 1973 with those born in 1925 The screening examination was performed from 4 30 to 7 p m BP was measured in the right arm in the sitting position after 5 min rest A rubber cuff 12 5 cm broad and 26 cm long connected to a mercury manometer was used Diastolic BP was determined as phase 5 i e when the sound disappeared Room temperature was 21–23°C Systolic and diastolic BP (SDP and DBP) were determined to the nearest 2 mmHg Height and weight were measured according to Rose and Blackburn (20) and weight was determined to the nearest 0 1 kg with a lever balance RBW was calculated as weight/(height −100) Date regarding history of myocardial infarction and stroke in parents and if the parents were alive their smoking habits and physical activities were taken from the postal questionnaire The participants were divided into non-smokers ex smokers and smokers Cigarette smokers were divided into those smoking 1–14 15–24 and ≥25 cigarettes a day Those who smoked other forms of tobacco than cigarettes were not included in the analysis The participants classified their degree of physical activity at work and during leisure time according to a 4 point scale (21 28) The validity of the scale has been tested against objective methods (10)

Standard methods have been used for calculation of mean S D and correlation coefficient The hypothesis of no difference in means between two groups was tested using Student s *t* test and for more than two groups using analysis of variance Differences were considered significant for $p<0.05$ Only 2 sided tests were used The hypothesis of linear correlation between two variables was tested by means of the correlation coefficient Linear relationships were presupposed for the multiple regression analysis The independent variables were chosen on the basis of previously known or probable relationships

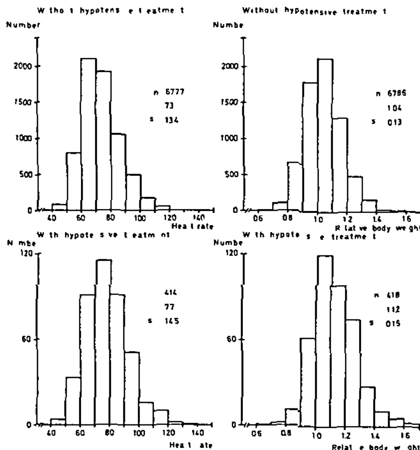


Fig 2 Heart rate and relative body weight in subjects with *and without hypotensive* treatment at the time of the screening examination

Whether or not addition of an independent variable added information was judged from the determination coefficient (r^2) The hypothesis of no differences in proportions be tween two groups was tested using the χ^2 test

RESULTS

Blood pressure

The distributions of SBP and DBP for subjects on and not on antihypertensive treatment are given in Fig 1 The distributions were all roughly normal with a slight skewness to the right No bimodality was seen in any of the distributions

The arbitrarily drawn BP limits for further man agement are given in Fig 1 SBP≥175 mmHg was found in 10% and DBP ≥115 mmHg in 6% of subjects not on hypotensive treatment

In the group on hypotensive treatment SBP ≥175 mmHg was found in 43% and DBP ≥115 mmHg in 30% The mean SBP and DBP were much higher in this group than in the group not on hypotensive treatment

Relationship between height weight and relative body weight

In order to examine whether RBW could be used as a common denominator of height and weight a multi ple linear regression analysis was performed The following equation was found

$$\mathrm{RBW} = \frac{-0\ 01200}{\pm 0\ 00007}\ \mathrm{height}\ \frac{+0\ 013000}{\pm 0\ 00004}\ \mathrm{weight} + 2\ 189$$

The multiple correlation coefficient was 0 97 and the determination coefficient 0 94 i e 94% of the varia tion in RBW could be explained by a linear function of height and weight The addition of age as an *independent variable in the regression analysis did* not increase the determination coefficient As RBW well described the relationship between height and weight it was used in the following analysis

Heart rate and relative body weight

The distributions of HR and RBW were roughly normally distributed with a slight skewness towards higher values both in subjects not on hypotensive

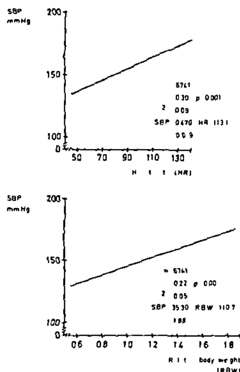


Fig 3 Relationship between heart rate (HR) and systolic blood pressure (*SBP*) and between relative body weight (*RBW*) and systolic blood pressure (*SBP*)

treatment and in those on such treatment (Fig 2) HR (p<0 01) and RBW (p<0 001) were higher in subjects on hypotensive treatment than in those thout

ationship between blood pressure and other variables

The linear correlation coefficients between the variables studied in subjects not on hypotensive treatment are given in Table I The following significant relationships (p<0 05) were found Age was negatively correlated to height The other variables including BP were not significantly correlated to age Height and weight were positively correlated RBW was positively correlated to SBP and DBP HR was positively correlated to SBP and DBP

The weak relationships between BP and HR and between BP and RBW are illustrated in Fig 3 Although significant positive linear correlations were found only 9% of the variation in SBP could be explained by a linear function of HR and only 5% by a linear function of RBW

BP and family history of myocardial infarction cerebrovascular disease and parental death

Mean SBP and DBP were calculated in subjects with and without family history of myocardial infarction and cerebrovascular disease and in subjects whose parents were dead or alive at the time of the screening examination (Table II) Subjects who stated that their father or mother had suffered myocardial infarction had significantly higher DBP Those who stated that their mother had had myocardial infarction also had higher SBP The absolute mean differences between those with and without family history of myocardial infarction did not however exceed 2 mmHg

Subjects who stated that their father and mother had suffered stroke had significantly higher SBP and DBP but the absolute differences were again small

Subjects whose father and mother were dead also had significantly higher SBP and DBP than those whose parents were alive at the time of the screening examination

Relationship between BP smoking habits and physical activity

Mean SBP and DBP were calculated for groups with different smoking habits and for groups with different physical activity at work and during leisure time

Table I *Correlation matrix*

	Age	Height	Weight	RBW	SBP	DBP	HR
Age	–						
Height	−0 403	–					
Weight	−0 112	0 449	–				
RBW	0 066	−0 075	0 830	–			
SBP	0 040	0 053	0 151	0 223	–		
DBP	−0 007	−0 057	0 211	0 287	0 732	–	
HR	−0 069	−0 142	0 026	0 114	0 295	0 224	–

Table II *BP in subjects with and without family history of myocardial infarction stroke and parental death Only subjects without hypotensive treatment are included*

	SBP			DBP			Level of statistical significance	
	n	*x*	*s*	*n*	*x*	*s*	SBP (*p*<)	DBP (*p*<)
Family history of myocardial infarction								
Father infarction	923	148	22 2	923	95	13 1	n s	0 001
Father not infarction	4 770	147	21 0	4 770	93	12 2		
Mother infarction	511	149	22 4	511	95	12 9	0 05	0 001
Mother not infarction	5 439	147	21 2	5 439	93	12 2		
Family history of stroke								
Father stroke	893	149	23 0	893	95	13 5	0 05	0 001
Father not stroke	4 813	147	20 8	4 813	93	12 1		
Mother stroke	1 029	150	21 7	1 029	95	12 8	0 001	0 001
Mother not stroke	4 982	147	21 2	4 982	93	12 3		
Parental death								
Father dead	5 152	148	21 3	5 152	94	12 4	0 005	0 01
Father not dead	1 366	146	21 0	1 366	93	12 0		
Mother dead	4 100	148	21 5	4 100	94	12 4	0 001	0 005
Mother not dead	2 540	146	20 9	2 540	93	12 3		

(Table III) Non smokers and ex smokers had the highest DBP and those who smoked more than 25 cigarettes a day had the lowest DBP There was a significant mean difference in DBP but not in SBP No significant differences in BP were found between groups with different physical activity at work and during leisure time Low physical activity at work and during leisure time (grade 1 according to a 4-point scale) was found more often in subjects on hypotensive treatment than in the remainder of the population (x^2=5 86 and 5 15 respectively $p<$ 0 025)

Table III *BP in groups with different smoking habits and different degrees of physical activity at work and during leisure time Only subjects without hypotensive treatment are included*

	SBP			DBP			Level of statistical significance	
	n	*x*	s_x	*n*	*x*	*s*	SBP (*p*)	DBP (*p*<)
Smoking habits								
Non smoker	1 964	148	21 8	1 964	95	12 5		
Ex smoker	1 392	148	20 8	1 392	95	12 3		
Smoker								
1–14 cig /d	2 235	147	21 1	2 235	93	12 5	n s	0 01
15–25 cig /d	1 013	147	21 0	1 013	92	11 9		
>25 cig /d	163	146	19 8	163	91	11 1		
Physical activity								
At work								
Degree 1	1 930	147	21 0	1 930	94	12 3		
Degree 2	2 658	148	21 4	2 658	94	12 5	n s	n s
Degree 3+4	2 101	148	21 3	2 101	94	12 2		
During leisure time								
Degree 1	1 713	147	21 4	1 713	94	12 7		
Degree 2	3 916	148	21 2	3 916	94	12 2	n s	n s
Degree 3+4	1 079	146	21 2	1 079	93	12 4		

DISCUSSION

We have chosen to study men within a narrow age span in order to minimize possible influences of age and sex on the variables studied The material has been drawn so that the results can be generalized to other male populations with a similar background This is acceptable if the non participation group does not differ substantially from the population studied Earlier epidemiological studies in our group have shown that the non participation group might differ in socioeconomic factors (26) and in mortality (30) The non participation rate in the present study was 25% Similar or even higher rates have been reported from some population studies (3 4 6 7) Other studies have however shown higher participation rates (15 26 27) The present study which is a primary preventive trial was designed so that it would be possible to carry it out on a larger scale if the results proved favourable Further efforts have therefore not been taken to get hold of those who did not answer two letters of invitation It has been supposed that those who were unwilling to come to a screening examination would also be reluctant to follow therapeutic regimens

The BP limits for further action SBP=175 or DBP=115 mmHg were chosen because a previous study had shown a high prevalence of hypertensive organ manifestations above these limits (26) The descriptive BP data are in good agreement with earlier studies in Goteborg showing that a majority f persons with hypertension were untreated and t a large part of those on treatment had high BP 29)

igher HR at higher BP has also been reported in another epidemiologic study in this country (11) Young persons with essential hypertension have been found to have increased cardiac output due to increased HR and/or increased stroke volume—a hyperkinetic circulation (12 14 22) Cardiac output has not been determined in the present study but the positive correlation between BP and HR is in accordance with a hyperkinetic circulation in hypertensive men even in this age group

The increase in RBW with increasing BP is consistent with the findings in previous studies (3 13 25 26) High RBW seems to be caused by obesity in most middle aged men (26) The positive correlation between RBW and BP might be explained by errors in the BP determination due to thick overarms in the obese subjects (9) A previous study (26) has however shown that other measures of hypertension such as eyeground changes and ECG changes increase with increasing weight

The results regarding family history of myocardial infarction, stroke and parental death must be seen against the background of the uncertainty attached to history taking with a questionnaire Data on parental age at death were not available Differences in the frequencies of myocardial infarction stroke and parental death might thus be caused by differences in parental age when the participants were born

Several workers have studied the relationship between BP and family history of myocardial infarction and stroke A study of 4 376 insurance examinations showed a small but statistically significant increase of cardiovascular diseases in parents of hypertensive subjects (8) Bedell Thomas and Cohen (1) studied relatives of 266 medical students and found that hypertension and heart disease were most prevalent among those whose parents had hypertension Stamler (25) studying males aged 40–59, found a slightly higher BP and a higher prevalence of hypertensive heart involvement in subjects with a family history of early cardiovascular disease Ostfeld and Paul (19) showed that men aged 40–55 with parents having hypertension or cerebrovascular disease had slightly higher BP than subjects without such a family history Previous studies have thus shown a weak but significant relationship between BP and family history of myocardial infarction and stroke Our results are in agreement with this

Genetic factors cannot be differentiated from environmental factors which complicates the interpretation of results A previous study has shown a relationship between BP and blood group markers indicating that genetic factors are of importance for determination of the BP level It was however not clear to what extent the variation in BP can be explained by genetic factors (18) Our findings are in good agreement with the theory of polygenic inheritance in hypertension The genes carrying the disposition for hypertension are according to this theory very widespread in the population and small differences might therefore be expected between groups with and without a family history of hypertensive complications (24)

Several authors have reported that the frequency of smokers decreases with increasing BP (13 26) In the present study there was no difference in RBW between groups with different smoking habits The lower BP in subjects smoking >25 cigarettes a day was thus not explained by a lower RBW In a recent

study Seltzer (23) has found that recent quitters displayed significant increases in SBP in all categories of weight change whereas the continuing smokers showed declining pressures with weight loss and increasing pressures with weight gain

The relationship between physical activity and BP has been little studied in large population samples Morris and Crawford (16) analysed the autopsies of 3 600 middle aged males and found that those who had had light work showed signs of hypertensive disease more often than those who had had heavy work Morris et al (17) also found that subjects with light work had a higher prevalence of hypertension than those with heavy work In the Framingham Study Dawber et al (6) found that low BP was associated with high physical activity in men aged 50–59 but not in younger men or in women In the present study no relationship was found between physical activity and BP The different results might be explained by differences in the populations studied The other studies included hypertensive subjects on hypotensive therapy whereas in the present study these subjects were analysed separately It is not possible to say whether the hypotensive treatment had induced the decrease in physical activity or whether low physical activity characterized those subjects even before the initiation of treatment

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Ectopic Pituitary Function

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ABSTRACT Two patients with acromegaly have been treated with hypophysectomy Because the disease was still active the patients were reoperated No pituitary tissue could be found at the second operations Besides the acromegaly one of the patients had diabetes mellitus (appeared after the first operation), Cushing s syndrome, probably ACTH dependent, and evidence of thyrotoxicosis In both patients extopic pituitary tissue was suspected One of the patients reacted with a normal fall in plasma growth hormone to growth hormone releasing inhibiting hormone Ectopic pituitary function should be suspected, if pituitary function is retained after a hypophysectomy

It is well recognized that even after a seemingly complete hypophysectomy varying degrees of pituitary function may be retained This is usually ascribed to failure in removing all glandular tissue from the sella The existence of ectopic pituitary is however well established although the functional significance of such aberrant tissue is uncertain In this paper two patients presenting with severe acromegaly after a proved total hypophysectomy are reported

CASE REPORTS

Case I

The patient was a 52 year-old female when first admitted in 1972 For the last 10 years her facial features hands and feet had enlarged and become increasingly coarse She had noticed excessive sweating and paresthesia in her hands There had been no headache or visual disturbances Her menstrual bleedings had ceased when she was 46 years old No other relevant symptoms were present The physical examination revealed obesity and a typical acromegalic appearance There was a discrete nodular goiter *Clinically the patient appeared euthyroid There* were no abnormal pigmentations hirsutism or acne

X ray examination revealed an enlarged sella but pneumoencephalography disclosed no suprasellar tumor Ophthalmological examination was normal BP was 130/90 mmHg There was no glucosuria but an oral glucose tolerance test (OGT) was slightly diabetic The laboratory findings are listed in Table I The urinary excretion of 17 ketogenic steroids (17 KGS) was rather high but at that time considered normal in view of the obesity

It was thought that an active acromegaly was present A wholly intrasellar pituitary adenoma was totally removed through a transfrontal craniotomy Histologically the adenoma was a mixed adenoma (chromophobe+eosinophil)

Following the routine of this Department the patient was discharged without any hormonal substitution She was followed up as out patient and readmitted 6 months after the operation for repeat studies (Table I) There was now a severe diabetes mellitus which surprisingly could be well controlled by dietary restrictions+metformin

The patient was readmitted again 4 months later Besides the results given in Table I a dexamethasone (DXM) test was carried out On DXM 2 mg daily for 3 days the urinary excretion of unconjugated cortisol fell from 278 to 95 μg/24 h and on DXM 8 mg daily the excretion fell to 34 μg/24 h

Clinically the patient had not changed Sweating and paresthesia were still present although the acromegalic facial features may have undergone slight regression Two *months later the clinical impression was that a still active* acromegaly was present At this time the 24 hour ^{131}I thyroid uptake was 34% and after administration of 100 μg triiodothyronine (T3) daily for 4 days 27% Serum protein-bound iodine (PBI) was slightly raised No thyrotropin (TSH) could be demonstrated before or after i v injection of thyrotropin-releasing hormone (TRH)

A diagnosis of a still active acromegaly together with a pituitary-dependent adrenal hypercorticism and diabetes was made Thyrotoxicosis was strongly suspected because of the non suppressible ^{131}I-uptake

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Table I *Results in patient 1*

	Normal values	1st operation Before	6 mo after	10 mo after	2nd operation Before	6 mo after	10 mo after
GH (OGT) (ng/ml) basal max min	<2 (min)	84–110	37–52	46–66	44–29	81–58	35–25
GH (IAT)[a] (ng/ml) basal max	>4 2 (max)		44–88	35–110		36–167	
Urinary pituitary gonadotrophins (mU/24 h)	>6.5	63	4	18		0	
17 KGS (mg/24 h)	<10	19 7	11 0	30 6	15 4	16 8	17 6
Plasma desoxycortisol at 4 h i v metyrapone test (μg/100 ml)	>3 0	8 9	9 5	11 9		11 6	3 5
Urinary unconjugated cortisol (μg/24 h)	<170			300			2 480
Serum thyroxine (μg/100 ml)	4 7–10 9	10 4	13 6	10 6	9 5	7 7	7 9
Plasma ACTH (pg/ml)	16–120					67	37

[a] IAT=i v arginine test (500 mg arginine monohydrochloride being infused over a 30 min period and serum GH being measured at 0 15 30 45 and 60 min)

It was decided to perform a repeat craniotomy as it was suspected that remnants of the pituitary might still be present in the sella However a completely empty fossa was found at operation It was not possible to obtain even microscopical fragments of pituitary tissue

The patient was studied 6 and 10 months after the second operation On the last admission she had been on chlorpromazine 25 q d and medroxyprogesterone 10 mg q d for 2 months without any appreciable effect After a trial of insulin because of increasing dysregulation the diabetes tolbutamide was given In addition to the given in Table I gonadotropin releasing hormone was injected i v and follicle stimulating hormone (FSH)+luteinizing hormone (LH) were measured No increase in either hormone occurred from the basal level of 3 mU/ml—a concentration far below the normal level The patient still had no menstrual bleedings

Six months after the second operation growth hormone releasing inhibiting hormone (GH RIH or SRIF) was given The results are shown in Fig 1

Case 2

The patient was a 45 year-old male He was first seen in 1970 In 1967 he had received X ray treatment because of an active acromegaly which was not arrested by this He therefore underwent a hypophysectomy in another hospi

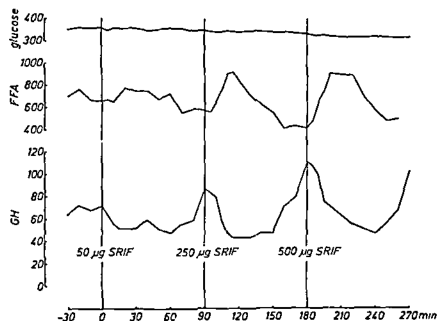


Fig 1 Plasma growth hormone (*GH*) (ng/ml) blood glucose (mg/100 ml) and free fatty acids (*FFA*) in plasma (μEq/l) after i v injection of growth hormone releasing inhibiting hormone (*SRIF* or *GH RIH*) in increasing doses (case 1)

tal in 1968 through a transsphenoidal approach Prior to the operation basal growth hormone (GH) concentration was 40 μg/ml with no suppression during hyperglycemia The function of the thyroid adrenals and gonads was judged normal from the various investigations performed

As symptoms of an active acromegaly persisted he was admitted to this Department in 1970 He was then on substitution with thyroxine and cortisone The basal GH concentration was now 55 ng/ml with a paradoxical rise to 64 during hyperglycemia At reoperation with a trans frontal approach the sella was found to be completely empty There was no suprasellar tumor Postoperatively no substitution was given The patient soon developed clinical signs of adrenal insufficiency and this was con firmed biochemically as the plasma cortisol was low (1 2 μg/100 ml)

Since then he has been followed up as out patient The condition has been excellent on cortisone substitution There has been no further acral growth or other manifesta tions of an active acromegaly The last investigations were performed 2½ years after the second operation At this time the basal GH concentration was 8 ng/ml falling to 4 ng/ml during hyperglycemia Besides cortisone testos terone was given because of clinical and biochemi cal evidence of testicular failure There have been no clinical or biochemical signs of hypothyroidism

DISCUSSION

In case I there can be no doubt that a considerable degree of pituitary function was retained after hypophysectomy with clinical evidence of a pro gressive acromegaly with elevated GH level in plas ma It is not entirely clear whether the adrenal hyperfunction was pituitary-dependent Cortico-tropin (ACTH) was not within the range usually seen in adrenal hyperplasia The substantial suppressibility of the excretion of unconjugated cortisol points to a pituitary dependence Plasma ACTH fluctuates widely and the plasma samples may have been drawn while the concentration was at a nadir The main point seems to be that appreci able amounts of ACTH could be demonstrated

The fact that no TSH could be found in plasma may readily be explained by the existence of a thyrotoxicosis or by the adrenal hyperfunction as corticosteroids are known to suppress the concen-tration of TSH and the effect of TRH (16) Thus it cannot be proved that the patient was incapable of secreting TSH On the other hand there can be no doubt that the secretion of pituitary gonadotropins was definitely invalidated

The flare-up of the diabetes mellitus following the first operation is not easily understood and can hardly be explained by the adrenal hyperfunction

In case 2 an undoubtedly active acromegaly persisted despite a hypophysectomy which a later operation showed was complete In this case the acromegaly regressed after the second operation although no pituitary tissue was removed

One should emphasize the well recognized fact that there is no definite relationship between the clinical severity of an acromegaly and the level of circulating GH (6) In both cases however the clinical symptoms after the first operation leave no doubt that a progressive acromegaly was still pres ent The most reasonable explanation in both cases seems to be that ectopic pituitary tissue was pres ent

The most common type of ectopic pituitary tissue seems to be the so called pharyngeal hypophysis in the roof of the naso pharynx first described by Erdheim in 1904 (4) The early literature has been well reviewed by Boyd (2)

The pharyngeal pituitary is apparently present in all or nearly all subjects (2 7 14 15) Cells thought to be secreting GH TSH and ACTH have been demonstrated as well as GH thyrotropin and pro lactin like activity (12)

The findings of McGrath (13) suggest that the pharyngeal pituitary may be under the influence of hypothalamic hormones as it was demonstrated that venous anastomoses exist between the capsule of the adenohypophysis and the tissue immediately adjacent to the pharyngeal hypophysis Intra sphenoidal pituitary tissue seems to be very rare (18)

Ectopic pituitary tissue should not be confused with extrasellar extension of an intrasellar neo plasm which is by no means rare (1 8 9) and which may be found though probably very seldom in the nasal cavity (10) or in the sphenoidal bone (5)

Tonnis et al (17) suggested that hypophysectomy might lead to a hypertrophy of the pharyngeal pituitary Muller (15) investigated this possibility and concluded that such compensatory hyper trophy did occur Van Buren and Bergenstahl (3) in their study however came to the opposite conclu sion

The possibility of functioning aberrant pituitary tissue should be considered in patients with signs of continuous pituitary secretion after hypophysec tomy In a forthcoming paper (11) it is shown that in a considerable number of patients with en docrinologically active pituitary adenomas normal or even excessive hormonal secretion is retained

after hypophysectomy These cases do often re present a major therapeutic problem It is thus of some interest that GH RIH does appear to have an effect on GH of ectopic origin GH RIH may in the future prove to be of value in the treatment of acromegaly unresponsive to conventional therapy

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A Rapid Screening Test for Cushing's Syndrome

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ABSTRACT A combination test consisting of two parts, a single dose of dexamethasone and a (1–24) ACTH injection, has been used for the diagnosis of Cushing's syndrome The test was performed by administering 1 mg dexamethasone orally at 11 p m on the first day and 25 IU synthetic ACTH i v at 8 a m on the second day Plasma cortisol was determined at 8 a m on the first and second day and 1 1/2 and 2 hours after the ACTH injection The test was performed in 33 patients with Cushing's syndrome and in 114 controls without adrenal disease After dexamethasone administration alone, cortisol values frequently overlapped between patients with Cushing's syndrome and controls The validity of the test was considerably improved by adding ACTH stimulation The test procedure is recommended as a screening test for Cushing s syndrome in ambulatory practice

The value of the ACTH suppression test with dexamethasone in the diagnosis of Cushing s syndrome is well established The classical test described by Liddle in 1960 (9) is probably the most widely used and best standardized However it has the disadvantage of being relatively time-consuming and requires accurate urinary collection for adequate assessment The introduction of fluorimetric methods for the determination of corticosteroids made it simpler to determine plasma cortisol and led to the development of tests in which plasma cortisol was determined after a single dose of dexamethasone (13 14)

Since 1966 we have used a suppression test in which plasma cortisol is determined before and after dexamethasone followed by a stimulation test using a single injection of synthetic tetracosactrin (1–24) ACTH (17) The aim of the present study was to find out whether this simple screening test could be used in the diagnosis of Cushing s syndrome of various origins

PATIENT MATERIAL

Group 1 comprised 33 patients with Cushing s syndrome constituting 66 % of all patients with this disease evaluated and treated at our Department between 1967 and 1974 The diagnosis was established by using the metyrapone test (10) and the classical dexamethasone suppression test (5 10) Twenty-seven of the 33 patients were considered to have bilateral adrenocortical hyperplasia due to excess pituitary ACTH Three of them exhibited radiological evidence of a pituitary tumour at the time of diagnosis and were submitted to transsphenoidal hypophysectomy In two patients a chromophobic adenoma was disclosed at surgery and another one had a pituitary adenoma that could not be further classified The operation was unsuccessful in one patient who was later referred for bilateral adrenalectomy

The ultimate therapy was bilateral adrenalectomy in 22 of the patients Adrenal hyperplasia was confirmed at the operation One patient who had a haemorrhage in one adrenal in connection with phlebography of the adrenals had a temporary remission after surgical removal of the other adrenal followed by irradiation of the pituitary One woman 19 years of age had a complete remission following such irradiation

Among the patients primarily treated by bilateral adrenalectomy two developed pituitary enlargement three and five years respectively after the operation

Five patients with adrenocortical adenomas had complete remissions after removal of the tumour One patient exhibited the classical clinical features of Cushing s syndrome on the basis of adrenocortical cancer

Group 2 comprised 114 subjects Some of these were healthy members of the hospital staff others were patients with various disorders such as obesity essential hypertension idiopathic hypertrichosis and diabetes mellitus Many of them were admitted to the hospital on the suspicion of Cushing s syndrome This diagnosis was excluded in all on clinical grounds and/or by the dexamethasone suppression test (0 5 mg × 4 for four days) Patients with diseases of the gastrointestinal tract with vomiting diarrhoea, or malabsorption were excluded as were all patients on oral contraceptives

METHODS

The subjects received 1 mg dexamethasone orally at 11 p m on the first day Next morning at 8 a m 25 IU (1–24) ACTH (synthetic tetracosactrin Synacthen® Ciba) were

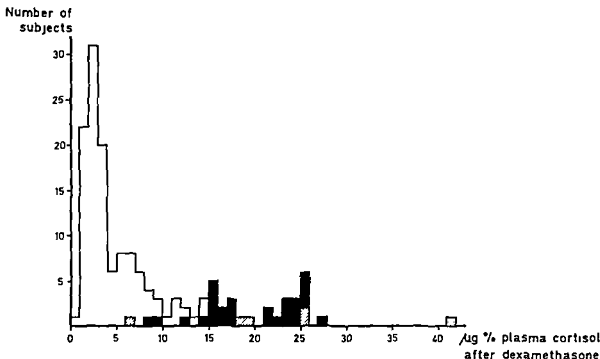


Fig 1 Plasma cortisol levels at 8 a m after administration of 1 mg dexamethasone at 11 p m the preceding evening □=controls ■=patients with adrenal hyperplasia ▨=patients with adrenal adenomas □=the patient with adrenocortical cancer

given i v Blood samples for cortisol determinations were collected on the first day at 8 a m on the second day at 8 m and 1 1 5 2 3 and 4 hours after the administration of) ACTH Later in the study cortisol was analysed *1 5 and 2 hours after (1–24) ACTH*
ne screening test was performed both on outpatients on inpatients Cortisol was measured in plasma using a fluorimetric method described by de Moor et al (11) and modified by Laurell (8)

RESULTS

Cortisol in plasma at 8 a m

The means of the basal cortisol levels in plasma at 8 a m in control subjects in patients with adrenal hyperplasia, and in patients with adrenal adenomas *were 18 6±0 6 22 5±1 1 and 18 9±2 8 μg/100 ml* respectively The corresponding S D were 6 0 5 7 and 6 2 μg/100 ml The highest level 33 3 μg/100 ml was found in the patient with adrenocortical cancer

Cortisol in plasma after suppression with 1 mg dexamethasone orally

The mean levels of cortisol in plasma 9 hours after 1 mg dexamethasone orally in controls patients with adrenocortical hyperplasia and patients with adrenal adenoma were 4 2±0 3 19 0±3 5 and 19 3±1 0 μg/100 ml respectively The highest value 41 7 μg/100 ml was again found in the patient with adrenocortical cancer As seen in Fig 1, none of the patients with Cushing s syndrome had cortisol values below 6 μg/100 ml and none of the controls had values above 15 μg/100 ml In five patients with Cushing s syndrome and in 26 controls the values were within the range of 6–15 μg/100 ml The Cushing patient with the lowest value 6 3 μg/100 ml had an adrenal adenoma *However in this patient urinary* 17 ketogenic steroids were suppressed with dexamethasone 0 5 mg four times daily In this respect she is unique for our whole material of patients with cortisol producing adrenal adenomas The remaining four patients with Cushing s *syndrome had adrenocortical hyperplasia with* pituitary dependence

The mean difference between the cortisol values before and after dexamethasone in the controls was 14 3±0 6 μg/100 ml ($p<0$ 001) The corresponding value for the group of patients with Cushing s *syndrome with adrenocortical hyperplasia was* 3 2±1 1 μg/100 ml The mean percentage decrease in the cortisol values from the basal level after dexamethasone suppression was 75 6±1 8 in the control group 11 2±5 3 in the patients with hyperplasia and

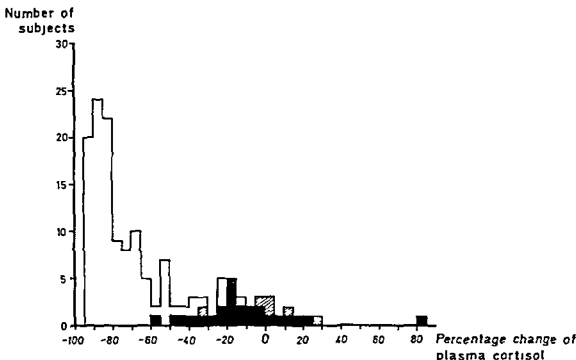


Fig 2 Percentage decrease of plasma cortisol from basal values after administration of 1 mg dexamethasone at 11 p m the preceding evening Symbols as in Fig 1

3 2±7 2 in the patients with adrenal adenomas It is seen from Fig 2 that this decrease was less than 60% in all the patients with Cushing s syndrome However in 17 of the controls the suppression was also decreased

When two criteria were combined i e a cortisol level after dexamethasone exceeding 6 μg and a decrease of less than 60% from basal value after dexamethasone 13 controls (11%) behaved like the patients with Cushing s syndrome

Cortisol in plasma after
(1-24) ACTH stimulation

The mean levels of cortisol in plasma before and after the i v injection of 0 25 mg tetracosactrin are shown in Figs 3 and 4 Significant elevations in the cortisol levels were obtained after one hour in the control subjects as well as in the patients with Cushing s syndrome with adrenocortical hyperplasia or adrenal adenomas In the control group the highest mean level was obtained after two hours but the increase was still significant after four hours Cortisol in plasma following (1-24) ACTH stimulation was higher in the group of patients with Cushing s syndrome than in the control group the difference between the controls and the patients with adrenocortical hyperplasia being highly significant ($p<0\ 001$) As regards the five patients with adrenal adenomas the variations in cortisol levels were too large to allow any conclusions The patient with adrenal carcinoma did not seem to respond to (1-24) ACTH stimulation the values before and after 1 1 5 2 3 and 4 hours being 41 7 46 3 47 1 32 9 and 28 7 μg/100 ml respectively

Since the cortisol levels after (1-24) ACTH injection were higher in the patients with Cushing s syndrome than in the control subjects this difference might be used as an additional diagnostic criterion As seen in Fig 5 the cortisol value after the (1-24) ACTH injection was above 30 μg/100 ml in all the patients with Cushing s syndrome Several of the controls also had values above 30 μg/100 ml Using the additional criterion that a patient with Cushing s syndrome shall also suppress less than 60% from basal value with dexamethasone only 10 controls (9%) behaved like the Cushing patients Fig 5 shows that a further separation was obtained by the equation $y=-0\ 6X+16$ where y represents the cortisol

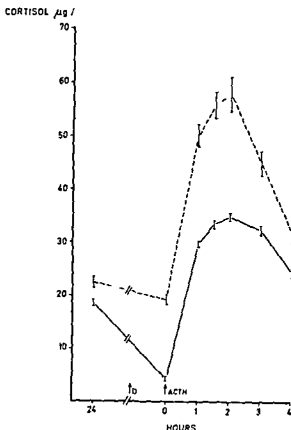


Fig 3 Plasma cortisol levels (±S E M) at 8 a m before after 1 mg dexamethasone orally and 1 1 5 2 3 and 4 s after an i v injection of 25 IU synthetic ACTH —controls (*n*=114) - - -=patients with pituitary adrenal hyperplasia (*n*=27) Dexamethasone (*D*) was given nine hours before (1-24) ACTH

level 1 1/2 hours after (1-24) ACTH stimulation and X the percentage change in cortisol level after dexamethasone administration *All* patients with Cushing s syndrome but only three controls (3%) had an intercept value (y+0 6X) above 16

DISCUSSION

It is well known that in the diagnosis of Cushing s syndrome single determinations of plasma cortisol levels are of greater value when performed after dexamethasone administration than under basal conditions (3 4 12) This is in accordance with the results in the present study However we found a considerable overlap with respect to the cortisol values after a single dose of dexamethasone between patients with Cushing s syndrome and those without

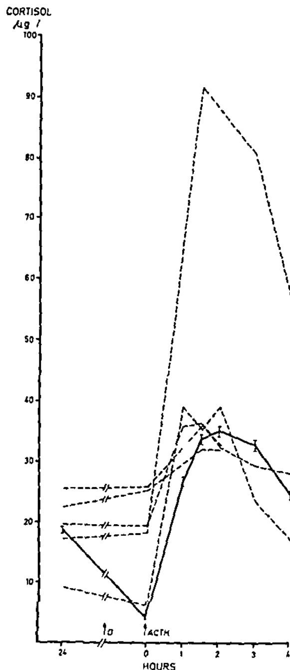


Fig 4 Plasma cortisol levels at 8 a m before and after 1 mg dexamethasone orally and 1 1 5 2 3 and 4 hours after an i v injection of 25 IU synthetic ACTH —=controls (±S E M) (*n*=114) - - -=individual curves for patients with adrenal adenomas Dexamethasone (*D*) was given nine hours before (1-24) ACTH

verified adrenal disease Utilizing the percentage decrease instead of the absolute values improved the usefulness of the suppression test All the patients with Cushing s syndrome had a decrease of plasma cortisol values of less than 60% On the other hand

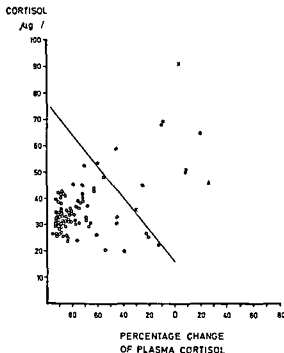


Fig 5 Scatter diagram between the percentage change in cortisol level after dexamethasone administration and the cortisol level 1 5 hours after ACTH stimulation in patients with Cushing s syndrome ($n=33$) and controls ($n=114$) O=controls ●=patients with adrenal hyperplasia ×=patients with adrenal adenoma ▲=the patient with adrenocortical cancer The equation of the solid line which separates patients with Cushing s syndrome from control subjects is $y=-0\ 6x+16$

15% of the control subjects showed the same reduced suppressibility The individuals with false positive results did not show any discrepancy concerning body weight or clinical symptoms The mean cortisol value after dexamethasone in 10 patients with body weights above 100 kg was approximately the same as the mean value for the whole control group

In general suppression tests are superior to stimulation tests in the diagnosis of Cushing s syndrome However in the present study the mean value of plasma cortisol after (1–24) ACTH stimulation was 80% higher in the patients with adrenal hyperplasia than in the control subjects Utilizing the information obtained by the ACTH stimulation in combination with the percentage suppressibility only 3% of the control subjects behaved like the patients with Cushing s syndrome Thus the addition of an (1-24) ACTH injection after a single dose of dexamethasone improved the validity of the test As expected this screening test could not distinguish patients with adrenal tumours from those with adrenal hyperplasia.

In several other studies (1 2 12–16 18 19) cortisol determinations after a single dose of dexamethasone has been described as a screening procedure for diagnosing Cushing s syndrome In 1965 Nugent et al (13) found that none of 17 patients with Cushing s syndrome had a cortisol level below 20 µg/100 ml after dexamethasone and therefore they were able to make a complete separation between patients and controls In the same year Pavlatos et al (14) in a smaller material obtained a complete separation between patients with and without Cushing s syndrome In later studies (3 15 16 19) however a frequent overlap in cortisol values has been demonstrated

A combination test with dexamethasone and ACTH has been used in a few studies (6 7) The conclusion was that the ACTH stimulation part did not give any further information but the materials were small and the combined information was not used The present study which comprised a larger material clearly showed the advantage of adding ACTH stimulation to the single-dose dexamethasone test The combination test used here is simple and suitable in ambulatory practice as a screening test in the diagnosis of Cushing s syndrome Any patient not fulfilling the two criteria used namely a decrease of less than 60% from basal cortisol value after dexamethasone administration and a cortisol value above 30 µg/100 ml 1 1/2 hours after (1–24) ACTH injection has to be further evaluated

In recent years routine methods have been developed for urinary free cortisol (2 3) and the determinations seem to be of great value in establishing excess production of cortisol However these determinations require accurate urinary collections for adequate assessment which is often difficult to achieve in ambulatory practice Therefore the test based on blood sampling has been most useful as a screening procedure for the diagnosis of Cushing s syndrome Furthermore it has been shown that the same test can serve as a screening procedure for diagnosing Addison s disease (17)

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A Study of Graves' Disease in Iceland

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ABSTRACT The incidence of Graves' disease and its response to treatment have been studied in Iceland where the main environmental factor possibly affecting Graves disease is a high iodine intake The incidence was found to be similar or lower to that reported for Europe The response to treatment with drugs and radioiodine does not differ from that found in other countries but recurrent hyperthyroidism after subtotal thyroidectomy is more common in Iceland

It is well known that the incidence of thyrotoxicosis and its response to treatment vary greatly between localities and various combinations of environmental genetic and treatment factors have been put forward to account for this A low incidence has been reported for the indigenous population of Africa (11 28 35) and differences in thyroid autoimmunity have been reported between African and Caucasian races (26)

The role of iodine in the pathogenesis of thyrotoxicosis is not clear The association of endemic goitre and toxic nodular goitre is generally accepted (8) and a temporary increase in the incidence of thyrotoxicosis after the introduction of iodine for goitre prophylaxis has been reported from various parts of the world (20 22 34) Thyrotoxicosis has been induced in euthyroid subjects with (39) and without (12) nodular goitres by high doses of iodine and recurrence of thyrotoxicosis after drug treatment has been associated with iodine repletion (2)

On the other hand high doses of iodine transiently inhibit the organification of intrathyroidal iodine hormone formation (40) and the release of hormone from the thyroid gland (38) in normal man In patients with underlying defects in hormone synthesis high doses of iodine can cause hypothyroidism (7)

The epidemiology of thyrotoxicosis and its response to treatment in a population on a chronically high iodine intake are of interest in this context Such a population is to be found in Iceland where the diet includes a large amount of fish Milk is also rich in iodine since cattle and other livestock are frequently fed on fish products (1) The mean plasma inorganic iodine for normal females in Iceland was found to be 3 times higher than for females in Aberdeen (10) and 2 5 times higher than for females in Glasgow (1) Simple goitre is very rare (10) and so is toxic nodular goitre

The present paper reports the results of a retrospective survey of patients with Graves disease in Iceland for the period 1938–67 and a follow-up study on samples of patients treated by surgery or radioiodine The Icelandic population is particularly well suited for epidemiological studies because of its smallness (200 000 in 1967) stability and centralized health services

MATERIAL AND METHODS

Patients

A study was made of records of patients with thyrotoxicosis in the surgical and medical departments of 6 hospitals for the period Jan 1938 to Dec 1967 Patients with this disease were not treated in other hospitals Up to 1960 the diagnosis was based on clinical symptoms and signs basal metabolic rate measurements as well as response to therapy After 1960 more exact diagnosis was possible with radioiodine tracer tests and protein bound iodine

All patients operated on had the excised tissue examined histologically Patients with toxic adenomas and toxic nodular goitre were excluded from the survey Patients were identified by name and date of birth and date of admission was noted Care was taken to count only first

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Table I *Results of a survey on patients with Graves disease in Iceland 1938–67*

Period	No of pats treated with Surgery	^{131}I	Drugs only	Total no of pats treated	$\frac{\text{Drugs only}}{\text{Total}} \times 100$ (%)	Mean population	Mean annual incidence per 1 000 inhabitants
1938–45	72		12	84	14	123 863	0 097
1946–50	21		49	70	70	138 504	0 1
1951–55	52		24	76	31	153 110	0 099
1956–60	77	3	37	117	32	170 167	0 138
1961–67	93	43	47	183	26	190 205	0 137
Total	315	46	169	530			

admissions Studies were also made of records of patients treated with radioiodine which were kept in the Isotope Department Landspitalinn and histology reports were studied in the Department of Pathology University of Iceland Reykjavik

Sampling of patients for follow up study

Surgical group Patients treated by subtotal thyroidectomy in the Surgical Department Landspitalinn during 1938–67 were selected for a follow up study The diagnosis of Graves disease was accepted for 237 patients 12 of whom had died and 3 could not be traced A letter was sent to the remaining 222 with a request to attend a follow up examination and 137 responded The patients were examined during the period April–Aug 1968 and some of them were under observation until Sept 1969 The mean period from operation to follow up was 13 6 years (range 1–30) There were 9 (6 6%) males in the eries The thyroidectomies were performed by 10 different surgeons but two of them undertook 80% The nant was usually assessed by eye to the nearest 0 5 g d the resected tissue weighed These data were available for 89 patients 82 of whom were operated by F Einarsson

Radioiodine group Radioiodine was first used in the treatment of thyrotoxicosis in 1960 and by Dec 1967 43 patients had received this treatment for Graves disease (Table I) A further 17 patients were treated up to Dec 1968 making a total of 60 available for a follow up study which was undertaken i July–Aug 1969 Eight patients had died but information was available on their thyroid status for most of the period from treatment to death Six patients were seen in 1968 with the surgical group Forty four patients attended a follow up examination Information was thus obtained on 52 patients 15 of whom had been treated for postoperative relapse (Table II) The radiation dose was calculated to give 7 000 rads to the thyroid (5) except in postoperative relapse where the dose was smaller

Methods of investigation

The clinical assessment of thyroid status was based on diagnostic indices for hyperthyroidism (9) and for hypothyroidism (4) Standard radioiodine tracer tests were used to measure 4-hour and 48 hour thyroid uptake and protein bound iodine 131 at 48 hours An ECG was obtained on all patients and ankle reflex time (6) when indicated Protein bound iodine 127 was measured in the Department of Chemical Pathology University of Aberdeen (Autoanalyzer methodology N56) and cholesterol in the Department of Clinical Biochemistry Landspitalinn Reykjavik Thyroid antibodies were measured by Professor J S Beck Aberdeen University Antibodies against thyroglobulin were measured by tanned red cell agglutination technique (TRC) and antibodies against thyroid cell cytoplasm (ACA) by immunofluorescent technique (17 36)

Methods of classification

Surgical group Hyperthyroidism At the time of review 13 patients were found to be thyrotoxic The diagnosis was based on all laboratory and clinical evidence as well as response to therapy Twenty patients presented with a history of a previously treated recurrence which was adequately documented responded convincingly to treatment and the diagnosis was accepted

Table II *Results of radioiodine treatment of Graves disease in Iceland 1960–68*

	No of pats	Mean age (y)	Average follow up (y)	Average initial ^{131}I dose (mCi)	No of pats requiring additional doses	Thyroid status at follow up: Thyrotoxic (n)	(%)	Euthyroid (n)	(%)	Hypothyroid (n)	(%)
Graves disease	37	52	4 1	4 8	7	1	2 8	29	78 3	7	18 9
Postop relapse	15	50	5 7	3 7	4	2	13 3	8	53 4	5	33 3

Hypothyroidism At the time of review 4 patients were classified as hypothyroid and 11 as suspect hypothyroid After a therapeutic trial 9 of these were classified as euthyroid and 2 as hypothyroid Seventeen patients were on thyroxine at the time of review The diagnosis of hypothyroidism was accepted for 5 Thyroxine was stopped for 10 of whom 7 were finally classified as euthyroid and 3 as hypothyroid Two patients were unclassified The therapeutic trials lasted 1–18 months and in judging the outcome the same methods and criteria were used as described by Hedley et al (15)

Statistical methods Differences in antibody frequency were tested by the χ^2 test or the exact Fischer test as appropriate (31) The differences in incidence of thyrotoxicosis were tested as differences in proportions (3) and the mean values for remnants were tested by the t test for means with unequal variances (3) Differences were considered to be significant if the probability of them arising by chance was less than $^1/_{20}$

RESULTS

Incidence Survey

The total number of patients treated was 530 (Table I) Subtotal thyroidectomy was performed on 315 patients 19 (6%) of whom required a second thyroidectomy for postoperative recurrent hyperthyroidism Patients who received medical treatment only numbered 169 They had no record of subsequent surgery or treatment with radioiodine and this is circumstantial evidence for a long lasting remission but no follow up was done on this group

The data in Table I have been analysed separately for 5 different periods since treatment policies or methods of diagnosis were different in these periods During 1938–46 medical treatment consisted of bed rest and administration of Lugol s solution The era of antithyroid drugs started in 1946 in Iceland and during 1946–50 70 patients received antithyroid drugs for several months or years but 21 were eventually referred for surgery Around 1950 treatment policy changed and fewer patients were given long term treatment with antithyroid drugs and referals for surgery after a short course of antithyroid drugs became common This change of policy was the result of an apprehension about the risk of agranulocytosis due to thiourea drugs (personal communication Professor S Samuelsson) This explains why 70% of the patients in the period 1946–50 were treated by drugs only compared to 31–26% in later periods Since all patients in the period 1946–50 were given a course of antithyroid drugs for several months the

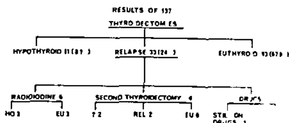


Fig 1 Results of partial thyroidectomy for Gra[illegible] ease in 137 Icelandic patients

colum $\frac{\text{Drugs only}}{\text{Total}} \times 100$ gives an estimate of the proportion of patients obtaining a long lasting remission for this period

In the period 1960–67 radioiodine tracer tests and protein bound iodine became available for diagnosis of thyroid state

The population in Iceland increased from 121 474 in Dec 1940 to 200 281 in Dec 1967 (24) and a mean population has been calculated for each period as well as the mean annual incidence (Table I) During the first three periods the incidence was 0 0[illegible]7 0 1 and 0 099 respectively and in the last two it was 0 138 and 0 137 respectively per 1 000 inhabitan[ts]

Follow up Studies

Surgical group

Clinical outcome The final classification of the 137 patients shows that 93 (67 9%) had remained euthyroid 11 (8 1%) became hypothyroid and 33 (24%) developed recurrent hyperthyroidism (Fig 1) The results of 10 second thyroidectomies for recurrent hyperthyroidism are similar to the first 6 became euthyroid 2 developed recurrent hyperthyroidism and 2 could not be classified (Fig. 1) (one had total thyroidectomy for suspected cancer another refused a therapeutic trial) Of the 33 patients with recurrent hyperthyroidism 11 relapsed within the first year after operation 9 were diagnosed between the first and fourth year 10 between years 4 to 20 and 3 patients were shown to be hyperthyroid more than 20 years after the original operation

Size of remnants and thyroid glands The mean estimated weight of the remnant for the recurrence group (4 66 g) was significantly larger than the remnant for the euthyroid group (3 73 g) ($p<0\ 01$) and for the hypothyroid group (3 78 g) ($p<0\ 01$) (Table III) Five patients had remnants of more than 6 g and all of them relapsed The mean size

Table III *Size of the thyroid gland and remnant in 89 patients with Graves disease treated by partial thyroidectomy*

	Diagnostic groups			
	Euthyroid (E) (n=60)	Recurrence (R) (n=22)	Hypothyroid (H) (n=7)	Significance of difference
Estimated remnant (mean±S E M)	3 73±0 09	4 66±0 27	3 78±0 008	E/R t=3 27
Range	2 0 –5 5	2 5 –7 7	3 0 –4 5	E/H t=3 1 p<0 01
Thyroid tissue removed plus estimated remnant (mean±S E M)	42 2±2 76	38 4±3 43	37 6±6 1	N S
Range	11–113	18–81	24–60	

of the thyroid glands was not significantly different in the three diagnostic groups (Table III)

Prevalence of thyroid antibodies Measurements of TRC and ACA antibodies were done in 119 patients The prevalence of TRC was 2 5 16 1 and 30% for the euthyroid recurrence and hypothyroid groups respectively the two last groups being significantly different from the euthyroid group The prevalence of ACA was 30 7 58 and 50% for the euthyroid recurrence and hypothyroid groups respectively and these differences did not reach levels of significance (Table IV)

—*Radioiodine group*

final classification of the 52 patients (Table II) s that of the 37 who had radioiodine as a first atment 29 (78 3%) had remained euthyroid 7 (18 9%) became hypothyroid and 1 (2 8%) was thyrotoxic Seven patients required two treatment doses Of the 15 patients who had radioiodine for postoperative relapse 8 (53 4%) had remained euthyroid 5 (33 3%) became hypothyroid and 2 (13 3%) were thyrotoxic Four patients required a second treatment dose

DISCUSSION

The annual admission rate of 0 09–0 137‰ for Graves disease in Iceland is probably a close estimate of the true incidence The standard of medical care and diagnosis of thyrotoxicosis was similar to that in Scandinavia during the study period and the policy was to admit all patients with thyrotoxicosis Information on the incidence of thyrotoxicosis in other countries is very scanty but what is known to the author is summarized in Table V An epidemic of thyrotoxicosis was noted in 1942–45 in Denmark Norway and France (19) but no such temporary increase was noted in Iceland over the 30-year period studied There is a trend towards an increased incidence after 1955 and a peak incidence occurred in 1958 0 19 per 1 000 inhabitants This trend may be partly due to improved diagnosis or it may reflect a true increase since the population changed from rural to predominantly urban during 1950–60 A higher incidence of thyrotoxicosis has been reported for urban populations (29, 30)

Differences in diagnostic methods and criteria may to some extent account for the difference found between countries but the results of the present study are in this respect comparable to Denmark because most Icelandic doctors during the period of study had postgraduate training in Denmark It is therefore noteworthy that the incidence of thyrotoxicosis in Denmark (19 37) discounting cases with toxic nodular goitre is very similar to that found for Graves disease in Iceland The incidence quoted for thyrotoxicosis in the Soviet Union (23) and Scotland (13) is higher than in Denmark or

Table IV *Prevalence of thyroid antibodies post thyroidectomy by thyroid status*

	TRC			ACA		
	+	–	%+	+	–	%+
Euthyroid (E)	2	76	2 5	24	54	30 7
Recurrence (R)	5	26	16 1	18	13	58
Hypothyroid (H)	3	7	30	5	5	50
Significance of difference	E/R p=0 029			E/R	x^2=2 25 N S	
	E/H p=0 018			E/H	x^2=0 25 N S	

Table V *Incidence of thyrotoxicosis in various countries*

Reference	Period	Country	Classification	Nodular goitre (%)	Annual incidence/1000 inhabitants
Greig (13)	Not given	West Scotland	Thyrotoxicosis	Not given	0
Iversen (19)	1938–42	Denmark (Copenhagen)	Thyrotoxicosis	12	0 1[illegible]
Iversen (19)	1942–45	Denmark (Copenhagen)	Thyrotoxicosis	23	0 [illegible] 4
Thommesen et al (37)	1964–68	Denmark (Soro amt)	Thyrotoxicosis	27	0 [illegible]
Levitt et al (23)	1963–69	Soviet Union	Thyrotoxicosis	Not given	0 354
Present study	1938–67	Iceland	Graves disease	0	0 09[illegible]

Iceland but the difference did not reach a level of significance

A prevalence of thyrotoxicosis of 0 8% has been reported for Japan (25) and the very high prevalence of 0.5–0 9% has been mentioned for Finland (21) thought to be due to the endemic goitre situation

The recurrence rate after subtotal thyroidectomy for Graves disease found in Iceland (24%) is higher than in most other countries (14) but the hypo thyroid rate (8 1%) is intermediate

Earlier studies have emphasized the association between thyroid antibodies and postoperative hypothyroidism (18) but the present study has shown that in Iceland thyroid antibodies are equally associated with postoperative hyperthyroidism and hypothyroidism This suggests that other factors may be more important in Iceland in determining the outcome of thyroidectomy Furthermore a comparative study of the frequencies of thyroid antibodies in North East Scotland and Iceland (17 36) showed a low frequency in the Icelandic population suggesting a low frequency of thyroiditis

A large remnant usually predisposes to post operative hyperthyroidism and a small remnant to hypothyroidism (16) The present study has shown that one of the main factors predisposing to post operative hyperthyroidism in Iceland is a large remnant but a remnant as small as 2–2 5 g was not associated with postoperative hypothyroidism However the outcome of thyroidectomy in Iceland cannot be accounted for simply by different methods of surgery since the mean remnant in this study (Table III) is in the middle or lower range of the most commonly reported remnant sizes (16) Nevertheless because of the high concentration of iodine in the Icelandic gland the Icelandic remnant may be left with greater iodine and hormonal stores at the time of surgery than most of its counterparts in other countries (36)

The combination of a low frequenc[illegible] of thyroiditis a high concentration of iodine in [illegible] thyroid gland and high iodine intake may acc[illegible]nt for the high recurrence rate in Iceland The [illegible] situation may explain the tendency to earl[illegible] lapse and the favourable response to a sec[illegible] thyroidectomy which is in marked contrast [illegible] reports (14 27) This suggests that result[illegible] improved by not leaving remnants in [illegible] 4 g

Because of the small number of patien[illegible] in th[illegible] radioiodine group and the short follow up [illegible] the results must be regarded as preli[illegible] [illegible] are however similar to those where the sam[illegible] do[illegible] age schedules have been used (32)

The present study provides only circumst[illegible] evidence about the results of drug treatment for Graves disease in Iceland The data in Table I suggest that possibly up to 70% of all patients treated by drugs in the period 1946–50 obtained a long lasting remission A more recent experience indicates that similar results have been obtained in the last 8 years in Iceland (personal communication S Th Gudmundsson Th Helgason) and this is comparable to results quoted by Solomon et al (33)

In conclusion the chronic high iodine intake in Iceland does not adversely affect the incidence of Graves disease or its response to treatment The high iodine intake is probably partly responsible for the high recurrence rate after subtotal thyroidectomy but results might be improved by more radical thyroidectomy

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Selected Psychosocial Variables in the Delay of Reaching the Coronary Care Unit

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ABSTRACT Sixty-one first admissions to a coronary care unit have been analysed regarding "delay period" from onset of chest pain to admission to the CCU in relation to psychosocial information, collected from the closest relative On the whole, psychosocial variables seemed to play a modest role in the determination of the delay period However, one "type A behavior" variable, inability to relax during leisure time, was related to a shorter delay period Young subjects tended to have a relatively short delay

A major problem in patients sustaining an acute myocardial infarction (AMI) is the high initial mortality outside hospitals One important means of reducing this would be to shorten the delay of admission to the coronary care unit (CCU) (2) This delay has been investigated by other groups (1 4 7 8 10 11) but studies of psychological and social variables which might be relevant to it are scant

It could be hypothesized that psychosocial variables relevant to the prognosis of coronary heart disease (CHD) may also be relevant to the delay period Such psychosocial variables are education (5) and type A behavior (a striving job-involved behavior reported to be excessively prevalent among premature CHD victims with a bad prognosis) (9) Furthermore the way people normally take care of their health when they develop symptoms of illness may be pertinent to the delay of admission after the onset of AMI symptoms

The present study describes the relations between delay of admission to a CCU after the onset of central chest pain and each one of three clusters of psychosocial variables namely formal educational level and documented experience or interest in medical issues (education variables) irritability (type A variables) and the way people handle their contacts with physicians and hospitals when they develop disease symptoms (illness behavior variables)

The best source of information regarding a person s normal behavior is in our opinion the closest available relative or friend who was therefore used as an informant for the present study Previous personal experience of CCU care presumably affects the delay behavior Therefore the present study was limited to first admissions

MATERIAL AND METHODS

Among all the patients admitted to the CCU at Serafimerlasarettet from Sept 1 1971 to Feb 28 197[illegible] 61 first admissions were accordingly selected for a study of factors related to length of delay Only patients with a *defined onset of chest pain were included* All the interviews concerning the circumstances to do with the disease onset were conducted by one of the investigators

The following selection criteria were used 1) Absence of previous care for a myocardial infarction 2) Availability—by telephone or personally—of a close relative who had taken an active part during the period in question i e from the start of chest pain to the CCU admission

It was considered unsuitable to interview relatives of patients who had died Therefore because in a few cases the relative was not available to us during the first 48 hours of the patient s CCU stay the patient s *survival* during this period was another limiting criterion

The relatives were interviewed (personally or by telephone) by one of two interviewers—a sociologist and a physician—who checked one another s five initial interviews in order to standardize the recording of answers The variables are listed in Table II The questions about irritability an inability to relax are similar to those used by Jenkins et al (6) for measuring type A behavior (1974) The diagnostic criteria of myocardial infarction used at Serafimerlasarettet have been presented elsewhere (12)

RESULTS

Table I shows the numerical distributions of the participating patients by sex and age

Table II shows the distributions of answers in relation to short (0–3 hours) and long (4 hours or

Table I *Sex and age distribution of 61 first admissions to the CCU*

Age (y)	MI		Observation cases	
	Males	Females	Males	Females
50	0	1	5	0
51–55	4	0	2	1
56–60	2	2	3	2
61–65	8	1	3	4
66–70	4	2	1	2
71–75	2	2	3	1
76–	1	2	2	1

more) delay respectively Two variables age <65 or ≥65 years and presence or absence of inability to relax during leisure time discriminated the two delay groups significantly from one another ($p<0.01$ and <0.05 respectively) Thus those who were under 65 and those reported by their closest relative to be unable to relax 'when there is plenty of time were those who tended to have a short delay of admission after the onset of symptoms Although none of the other variables were able to discriminate the groups significantly from one another there was a tendency for the patients whose closest relative

Table II *Individual variables in relation to short (0–3 hours) and long delay (4 hours or more)* $n=61$ (occasional subjects were unable to answer certain questions)

	0–3 h	4 h or more	
Age (y)			
<65	18	15	
≥65	7	21	$\chi^2=5.47$ $p<0.01$
Patient's formal education			
Elementary school	15	21	
More than elementary school	10	15	$\chi^2=0.02$ N S
Close relative's formal education			
Elementary school	12	24	
More than elementary school	13	12	$\chi^2=2.13$ N S
Patient's experience of nursing			
None	21	30	
me	4	6	Not tested
relative's experience rsing			
ne	18	27	
Some	7	9	$\chi^2=0.07$ N S
Sees most TV programmes			
Yes	9	15	
No	16	20	$\chi^2=0.29$ N S
Sees most TV programmes about heart			
Yes	9	15	
No	16	20	χ^2 0.29 N S
Frequent studies of diseases (reading medical books)			
Yes	4	2	
No	21	33	Not tested
Irritated by slow persons			
Yes	11	15	
No	14	20	χ^2 0.01 N S
Irritated in queues			
Yes	6	6	
No	19	28	$\chi^2=0.36$ N S
Inability to relax during leisure			
Yes	15	12	
No	10	24	$\chi^2=4.25$ $p<0.05$
Raises voice unnecessarily			
Yes	4	6	
No	21	30	Not tested
Normally stays at home when having a cold			
Yes	7	7	
No	17	29	$\chi^2=0.76$ N S
Consults physician at least 3 times per year			
Yes	10	17	
No	15	19	$\chi^2=0.31$ N S
Would normally participate in free health examination			
Yes	24	29	
No	1	7	Not tested
Would normally consult physician for minor illness			
Yes	14	16	
No	11	20	$\chi^2=0.79$ N S
Avoids doctors			
No	24	28	
Yes	1	8	Not tested
Avoids all pills			
No	13	13	
Yes	12	21	$\chi^2=1.11$ N S
Takes sleeping pill now and then			
Yes	9	9	
No	16	25	$\chi^2=0.62$ N S
Takes sedative now and then			
Yes	9	7	
No	16	26	$\chi^2=1.56$ N S
Takes sleeping pills daily			
Yes	3	6	
No	22	27	Not tested
Takes sedatives daily			
Yes	3	3	
No	22	30	Not tested

Table III *Inability to relax during leisure time in relation to short (0–3 hours) and long (4 hours or more) delay in different diagnostic groups*

	0–3 h	4 h or more
MI males		
Yes	4	5
No	2	10
MI females		
Yes	4	4
No	1	1
Obs cases males		
Yes	4	3
No	5	7
Obs cases females		
Yes	3	1
No	2	5

had a high level of education to have a shorter delay and generally speaking a tendency for those patients who reportedly avoided physicians and drugs to have a longer delay

Table III shows diagnostic group and inability to relax during leisure The relation between inability to relax and short delay was consistent across the four subgroups (male and female MIs and observation cases) except of the female MIs of whom 80% were reported to be unable to relax

DISCUSSION

A study of this nature suffers by definition from the fact that only arrivers to the CCU are investigated and not those who did not come the latter being in fact the target population Inability to relax when there is plenty of time was shown to be related to shorter delay It is possible that a person who is normally to be considered hyperactive will speed up surrounding persons in the various phases of the delay The somewhat longer average delay of older vs younger subjects demonstrated here was not found in a previous study from our Department (3) The discrepancy may be explained by the fact that *readmissions* as well as *first admissions* were included in the previous study

There seemed to be no other strong relations between observed psychosocial characteristics and the delay period Thus other patient characteristics such as formal school education medical education and documented interest in Medicine seemed to be of minor or no significance for the delay period On the whole it may be concluded that the influence of psychosocial factors on the delay has been small in the studied sample Nevertheless the pr u study from our Department has demons larger proportion than expected of very short d (<2 hours) in subjects who had been admitted to a CCU Thus at least th medical education may influence the del (3) There was also a tendency although significant, for patients with a close highly cated relative to have a shorter delay It is p that medical education may be of greater significance when it is held by close relatives than when it is possessed by the patient himself

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Acta med scand Vol 198 pp 319–325 1975

Cutaneous Reactions of the Extremities of Diabetics to Local Thermal Trauma

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ABSTRACT An earlier report described cutaneous lesions consisting of erythema with or without *necrosis, on the legs and/or feet of elderly diabetics* and the cause was suggested to be an altered reaction to precipitating factors such as cardiac decompensation The present investigation concerns the cutaneous reactions to traumatization with local heat or cold to the skin of legs and forearms of 35 diabetics and 25 controls Petechiae within the area of traumatization with either heat or cold were observed more often in diabetics than in controls They occurred more frequently on the legs than on the forearms Among the controls, petechiae were observed only in those over 50 years of age and only on the legs In the diabetics under 50, petechiae were almost always observed when the duration of diabetes was 10 years or more but seldom in young patients with diabetes of short duration The duration of diabetes was not significantly related to the occurrence of petechiae in diabetics over 50 In these diabetics moreover, petechiae developed after traumatization with heat of a lower temperature than that which caused petechiae to appear in corresponding controls The initial skin lesions in dermopathia diabetica (Melin) have a reddened border The skin of *the legs of some of the diabetics developed an intensely* reddened border round the area of experimental heat or cold traumatization These patients were either elderly diabetics or younger patients with diabetes of long duration Each of them had dermopathia diabetica and each developed atrophic circumscribed skin lesions on the site of traumatization Nineteen diabetics had dermopathia diabetica and 16 of them developed atrophic circumscribed skin lesions on the site of traumatization lesions which were never seen in the controls Thus diabetics differ from controls in their reaction to a certain thermal trauma The possible reasons for this altered reaction are discussed

The author has earlier (16) described cutaneous lesions consisting of areas of erythema with or without necrosis on the legs and/or feet of older diabetics It was suggested that these lesions were due to an altered reaction in diabetics to various precipitating factors chiefly cardiac decompensation It was therefore considered of interest to investigate whether or not the skin of diabetics compared to that of controls has an altered reaction to experimentally induced trauma To the author s knowledge there are no systematic experimental studies of the possible differences between diabetics and non-diabetics with respect to the response to cutaneous traumatization Both heat and cold were used in producing the injuries to small areas on the skin of legs and forearms

The effects of experimental local thermal traumatization to the skin of humans and animals have been studied by Leach et al (14) Lewis and Love (15) and others The lesions they described are generally those seen clinically as a result of burns i e erythema blisters and destruction of the skin (21 22 23) Local application of heat or cold may also cause capillary lesions with increased permeability and small local haemorrhages (9 24) The clinical observation that diabetic patients with gynecological cancer were less tolerant to radiation than non-diabetics stimulated Gunn et al (12) to undertake an experimental investigation which demonstrated that radiation exposure produced more severe skin lesions in alloxan diabetic rats than in non-diabetic controls However they did not report any results concerning the importance of the duration of diabetes In the present study the age of the patients as well as the duration of diabetes were recorded in order to be able to assess the possible effects of these factors on the reaction to trauma

METHODS

Local heat traumatization was performed by placing the end surface of an electrically heated right cylindrical brass

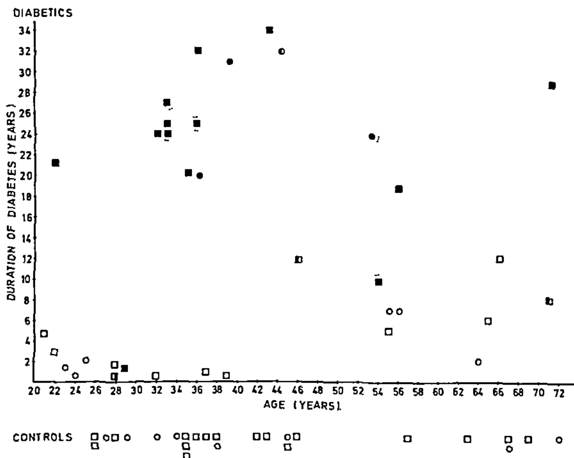


Fig 1 Age and sex of the diabetics and controls For the diabetics duration of the disease occurrence of dermopathia diabetica before traumatization development of an intensely red border and atrophic circumscribed skin lesions after traumatization of the skin of the legs with heat O=female □=male ◐ ◧=spontaneous dermopathia diabetica ◑ ◨=atrophic circumscribed skin lesions following the traumatization ● ■=spontaneous dermopathia diabetica and atrophic circumscribed skin lesions following the traumatization =intensely red border surrounding the area of traumatization

rod 8 mm in diameter in contact with the skin for 5 sec The pressure on the skin was the weight of the rod alone The temperature of the rod was 60° or 55°C The temperature of the part of the brass rod which rested against the skin could be controlled with an accuracy of <±1 C

Local trauma with cold was performed by applying the plane end of a cylindrical pencil of solid carbon dioxide 8 mm in diameter to the surface of the skin for 5 sec A constant pressure on the skin was achieved by means of a spring device

Both the patients and the controls displayed a positive attitude to the experimental trauma and willingly came to each of the recall visits even though for some of them this meant a journey of as much as 130 km each time Experience showed that no risks were involved for the patients No anaesthesia was used

The skin sites selected for experimental traumatization were approximately in the middle of the extensor side of the lower leg and approximately in the middle of the extensor side of the forearm The traumatized areas have been observed at 1 7 14 21 60–80 120–140 and 200–220 days Any skin changes were photographed in color

Differences between groups were tested using Fischer s exact probability test $p<0.05$ was chosen as the level for statistical significance

MATERIAL

Thermal traumatization of the skin of the legs and forearms was performed in 35 diabetics and 25 controls All subjects were ambulatory The patients were all in good general health well regulated in their diabetes and none had demonstrable leg edema The controls were persons on the medical staff of the Department of Medicine with the exception of five of the oldest controls who were out patients with no serious diseases Glucose tolerance tests were not performed in the controls with one exception—a person with pronounced local cutaneous reactions Data concerning the material are given in Table I and Fig 1

Table I *Distribution of the subjects with regard to age and of the diabetics with respect to the duration of diabetes treatment and possible complications*

	Diabetics				Controls	
Age group (y)	<50		≥50		<50	≥50
Duration of diabetes (y)	<10	≥10	<10	≥10		
Diabetics and controls (*n*)	11	13	6	5	19	6
Insulin treated pats (*n*)	11	13	0	4	0	0
Retinopathy (*n*)	0	12	1	3	0	0
Nephropathy (*n*)	0	5	1	2	0	0
Neuropathy (*n*)	0	11	3	5	0	0
Dermopathia diabetica (Melin) (*n*)						
Legs	1	13	1	4	0	0
Forearms	0	3	0	0	0	0
Mean age (y)	28 0	35 6	61 2	60 0	36 7	65 8
Mean duration of diabetes (y)	1 4	25 2	5 8	18 8	0	0

RESULTS

The majority of the subjects investigated developed erythema and blisters at the sites of thermal trauma and none of them displayed necrosis of the skin Many of those investigated developed small haemorrhages (petechiae) in the area of traumatization A number of the diabetics developed an intensely reddened border surrounding the area of traumatization A large number eventually developed atrophic circumscribed skin lesions

Blisters Observation twenty four hours after the experimental trauma disclosed a blister an erythema or no reaction at all If a blister was present it was often impossible to determine whether or not an erythema had developed as well because the blisters were often opaque Consequently no information is given about the presence or not of erythema At later observations the blisters had transformed into eschars which generally were transparent Blisters occurred significantly more frequently in younger compared to older controls (legs heat 60°C and cold $p<0\ 05$ and $p<0\ 01$ respectively) and in younger compared to older diabetics (legs heat 60°C $p<0\ 002$) There was no significant difference in this respect between diabetics and controls from the same age group Within the different groups there were no significant differences between the incidence of blisters on the legs and forearms The duration of diabetes was of no significance

Table II *Frequency of the occurrence of petechiae in the area of traumatization some time during the observation period*

	Diabetics				Controls	
Age group (y)	<50		≥50		<50	≥50
Duration of diabetes (y)	<10	≥10	<10	≥10		
Heat 60°C						
Legs	5/11	12/13	6/6	5/5	0/19	4/6
Forearms	1/11	0/13	2/6	1/5	0/19	0/6
Heat 55°C						
Legs	5/11	11/13	2/6	4/5	0/19	0/6
Forearms	0/11	1/13	1/6	1/5	0/19	0/6
Cold						
Legs	4/11	11/12*	6/6	5/5	0/17	4/6
Forearms	0/11	1/12	3/6	1/5	0/17	0/6

* Non transparent eschars at the site of traumatization prevented adequate observation in one of the persons investigated

Table III *Frequency of an intensely reddened border round the area of traumatization (legs heat 60°C and cold)*

	Diabetics				Controls	
Age group (y)	<50		≥50		<50	≥50
Duration of diabetes (y)	<10	≥10	<10	≥10		
Heat 60°C	0/11	5/13	0/6	3/5	0/19	0/6
Cold	0/11	8/12	0/6	1/5	0/17	0/6

Petechiae On the areas traumatized with either heat or cold petechiae were observed in both diabetics and controls (Table II and Fig 2) Petechiae were often observed for the first time 7 days after traumatization and thus appeared later than did the blisters Petechiae were observed approximately as often at sites traumatized with heat 60°C as at those traumatized with cold They were seen less often at sites traumatized with heat 55°C Petechiae occurred more often and were more abundant on the legs than on the forearms Petechiae were observed more often in diabetics than in controls Among the controls petechiae were only observed in those over 50 years of age only on the legs and only after traumatization with heat 60°C or cold not after traumatization with heat 55°C The petechiae in controls were always solitary with one exception a man of 69 who had abundant petechiae at the site of traumatization both with heat 60°C and with cold This person had a diabetic type of glucose tolerance test

Petechiae were significantly more common among diabetics under 50 years of age compared to controls of corresponding age (legs heat and cold) The difference between diabetics over 50 and corresponding controls was not significant In young diabetics the occurrence of petechiae was related to the duration of diabetes being significantly more common among those with long duration (legs cold $p<0.05$) In diabetics the petechiae were more abundant and were often confluent

Intensely reddened border round the area of traumatization Some persons presented a zone of intense redness (Table III Figs 1 and 3) This was never seen as early as 24 h after the traumatization but was generally manifest by 7 days This border remained for varying lengths of time from one week up to four months It occurred only on the legs and only after traumatization with heat 60°C or cold It was seen only in diabetics young and old and among these only in persons who had had the disease

Table IV *Frequencies of dermopathia diabetica before the traumatization and of the development of atrophic circumscribed skin lesions caused by the traumatization (heat 60°C and cold)*

	Diabetics				Controls	
Age group (y)	<50		≥50		<50	≥50
Duration of diabetes (y)	<10	≥10	<10	≥10		
Dermopathia diabetica before the traumatization						
Legs	1/11	13/13	1/6	4/5	0/19	0/6
Forearms	0/11	3/13	0/6	0/5	0/19	0/6
Atrophic circumscribed skin lesions caused by the traumatization						
Heat 60°C						
Legs	2/11	11/13	0/6	4/5	0/19	0/6
Forearms	0/11	3/13	0/6	0/5	0/19	0/6
Cold						
Legs	1/11	11/12	0/6	3/5	0/17	0/6
Forearms	0/11	1/12	0/6	0/5	0/17	0/6



2



3

4

Fig 2 Male 65 years old diabetes of 5 years duration Petechiae in the area of traumatization 7 days after local application of heat 60 C on the leg

Fig 3 Male 33 years old diabetes of 5 years duration Intensely reddened border round the area of traumatization 21 days after local application of solid carbon dioxide on the leg

Fig 4 The same patient as in Fig. 3 Atrophic circumscribed skin lesion on the area of traumatization 7 months after local application of heat 60 C on the left leg and cold on the right leg

for ten years or more and who also had dermopathia diabetica This intensely reddened border resembled that seen in the initial skin lesion of dermopathia diabetica (16)

Atrophic circumscribed skin lesions (19) Nineteen of the 35 diabetics had dermopathia diabetica on the lower extremities and 3 on the upper extremities (Table I) After traumatization on the skin of the legs with heat 60°C or with cold 16 of these 19 patients developed atrophic circumscribed skin lesions on the area of traumatization on the legs (Table IV Figs 1 and 4) Only one diabetic without dermopathia diabetica developed atrophic circumscribed skin lesions as a result of the experimental trauma The difference is significant ($p<0\ 002$) The interval between traumatization and the appearance of these skin lesions was 3 months or more As a rule *the lesions were pigmented and could not be* distinguished from those observed in these patients with dermopathia diabetica

None of the controls developed atrophic circumscribed skin lesions as a result of the traumatization All diabetics with an intensely reddened border round the area of traumatization developed atrophic circumscribed skin lesions The three patients with dermopathia diabetica on the forearms developed atrophic circumscribed skin lesions on the forearms as a result of the experimental traumatization with heat 60°C and one of them developed such a lesion after traumatization with cold

There were no obvious qualitative differences between the skin reactions to the local application of heat and cold respectively

DISCUSSION

In an earlier paper (16) the present author suggested that cutaneous lesions consisting of areas of erythema with or without necrosis of the legs and/or feet of elderly diabetics are due to a qualitatively or quantitatively altered reaction to precipitating factors such as cardiac decompensation with or without edema of the legs This is supported by the present results which shows that diabetics or certain groups of diabetics have an altered reaction to traumatization of the skin by heat or cold This was true for the occurrence of petechiae in the area of traumatization as well as for the development of reddened border zones and atrophic circumscribed skin lesions The latter two reactions were seen only in diabetics

From a physical point of view the thermal traumatization of the skin was identical in the diabetics and the controls However this does not imply biological identity For example the humidity of the skin may influence the relationship between the physical trauma and its biological intensity The presence of neuropathy may reduce perspiration and thus increase the biological intensity of the heat If a decreased skin humidity increases the biological effect of heat traumatization it ought to decrease the biological intensity of cold traumatization In this connection it should be noted that the results in diabetics and controls were similar concerning traumatization with heat 60°C and with cold

It is well known that dermopathia diabetica is localized as a rule to the lower extremities but may occur on the upper extremities as well (7 8) Three *of the diabetics in the present investigation had* dermopathia diabetica not only on the legs but also on the forearms These patients developed atrophic circumscribed skin lesions on the forearms in response to the thermal trauma They had no clinical signs of neuropathy in the upper extremities

Erythema with or without necrosis on the legs and/or feet has been seen in elderly people (16) This may partly be explained by the fact that precipitating factors such as cardiac decompensation are more common among older age groups Since the publication of the above mentioned article (16) these lesions have also been observed in three young individuals with diabetes of long duration and edema of the legs due to diabetic nephropathy

It is known that diabetic microangiopathy (1 25) and diabetic neuropathy (11 20) occur in the upper as well as in the lower extremities though they are more pronounced in the latter The altered cutaneous reactions observed in the present investigation were more pronounced in elderly diabetics and in juvenile diabetics with a long duration of the disease groups of patients specifically known to have microangiopathy These reactions were more pronounced in the lower extremities where microangiopathy is most pronounced and where diabetic neuropathy is common

The presence of diabetic microangiopathy would likely impede the passage of oxygen from the capillary blood to tissue cells as well as the passage of metabolites in the opposite direction The presence of neuropathy impairs the capacity to vary the blood circulation to the skin area in question (10 20) The presence of edema may also impair the passage

of oxygen and metabolites or bring about a compression of the small vessels and contribute to the development of the skin lesions described in the earlier paper (16) As is well known varicose leg ulcers for example hardly ever heal as long as edema is present

The altered reaction to traumatization of the skin observed in patients with juvenile diabetes of short duration might be due to the possible occurrence of an earlier latent diabetes with secondary microangiopathy The fact that an altered skin reaction to traumatization was not demonstrated as a rule in young patients with juvenile diabetes of short duration is evidence that the diabetic derangement per se is not of great importance for these altered reactions

In controls petechiae were observed in the area of traumatization only on the legs of elderly subjects The capillary basement membrane is normally thicker in the lower than in the upper extremities even in non diabetics (27) the thickness increasing with age (13) The difference in reaction between younger and older non diabetics and between the lower and upper extremities might thus be explained by these anatomical differences and their variation with age

The author also studied patients with familial amyloidosis and polyneuropathy (2 4) with respect to the occurrence of skin lesions (17) These patients often have many characteristic lesions localized to feet and lower legs The lesions are similar in respects to those which are common in diabetes of long duration or maturity onset abetes The author also studied the local cutaneous reactions to thermal traumatization of the forearms and legs of the amyloidosis patients Petechiae occurred within the area of traumatization with either heat or cold more often in these patients than in controls At the site of traumatization the patients often developed atrophic circumscribed skin lesions similar to those described by Melin (19) These amyloidosis patients with polyneuropathy have a decreased capacity to vary blood perfusion in the distal extremities (3) There are also amyloid deposits in the walls of the small blood vessels (5 6) The author suggested that the altered reaction to local traumatization in these patients as well as in diabetics is due to impaired transport from the blood to tissue cells It is noteworthy that the patients with familial amlyoidosis and polyneuropathy were not diabetic The local cutaneous reaction to thermal traumatization was also studied on the ears of rats with alloxan diabetes of various duration and was compared to that in controls (18) The local cutaneous reaction was observed as erythema at the site of traumatization Rats with alloxan diabetes of long duration clearly had a more pronounced reaction than corresponding controls Age and the diabetic metabolic derangement per se were also of importance for the appearance of more pronounced erythemas These findings strongly resemble those of the present investigation of diabetics

It is well known that burns often cause severe skin lesions on the lower extremities of diabetics This has been related to decreased sensibility caused by diabetic neuropathy (26) In diabetics the altered cutaneous reaction of the lower legs and feet might be of decisive importance for the development of skin lesions caused by traumatization, e g burns

ACKNOWLEDGEMENTS

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Announcements

The XIII International Congress of Internal Medicine organized by the Finnish Society of Internal Medicine and sponsored by the International Society of Internal Medicine will be held in Helsinki Finland Aug 15–19 1976

Preliminary scientific program Main sessions The role of bile acids in clinical medicine Immunosuppressive therapy Iatrogenic disease Prevention of ischemic heart disease Parallel sessions Hypothalamic pituitary system in internal medicine Diagnostic aspects of isotopes in medicine Etiology and pathogenesis of connective tissue disease Factors involved in the treatment of end stage uremia Septicemia Prostaglandins in internal medicine Viral infections in clinical medicine Medical aspects of the Finnish sauna

Free papers sessions Detailed instructions for submitting the abstracts of offered papers will be available from the Secretariat Deadline March 31 1976

Further information The Secretariat XIII International Congress of Internal Medicine P O Box 3 SF 00291 Helsinki 29 Finland

he International Prize for Modern Nutrition of Sfr will be awarded in Sept 1976 for recent rch work on the subject *Nutrition and immu* gy by the Central Union of Swiss Milk Producers Berne Switzerland to a scientist from one of the following countries Argentina Australia Austria Belgium Brazil Bulgaria Canada Czechoslovakia Denmark Finland France India Ireland Israel Italy Japan Kenya Luxemburg the Netherlands New Zealand Norway Poland South Africa Spain Sweden Switzerland United Kingdom U S S R West Germany

Applications to the President of the jury Professor M Demole 4 chemin Castoldi CH 1208 Genève Switzerland until Jan 31 1976 with 3 copies of a) curriculum vitae b) list of works c) reprints of 2 or 3 papers on the theme of the prize published in the last 5 years (no type written papers) These documents should be written in English French or German or should be accompanied by a translation into one of these 3 languages (They will not be returned to the authors)

World Congress on Progress in Vascular Diseases will be held under the auspices of the Western India Chapter of the American College of Chest Physicians in Bombay India March 19–21 1976

Information Dr M P Anand 4 Narendra Bhuvan Warden Road Bombay – 400026 India

2nd International Symposium on Acute Non traumatic Injury of the Lung will be held under the auspices of Tel Aviv University in Tel Aviv Israel April 26–29 1976

Information Organizing Committee of the 2nd Internat Symposium on Acute Non traumatic Injury of the Lung Meir Hospital Kfar Saba Israel

An IAEA Symposium entitled Medical Radionuclide Imaging will be held in Los Angeles California USA Oct 25–29 1976

Organizers International Atomic Energy Agency P O Box 590 A 1011 Vienna Austria

Scientific Secretaries L J Johansson and T Munkner Medical Applications Section

Further information and forms to accompany abstracts of papers intended for presentation at the Symposium may be obtained from national authorities for atomic energy matters Abstracts must be submitted through these authorities so as to reach the International Atomic Energy agency before May 21 1976

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Hydantoin Derivatives and Malignancies of the Haemopoietic System

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ABSTRACT Two patients are described who developed malignant lymphoma (lymphosarcoma) after diphenylhydantoin therapy because of epilepsy Malignant lymphoma in a few patients receiving this medication has been described earlier The literature has been reviewed and discussed recently by Rausing and Trell (2)

It is well known that administration of derivatives of hydantoin may be followed by various side effects One of them is swelling of superficial lymph nodes Such enlarged lymph nodes have been examined histologically by among others Saltzstein and Ackerman (3) and Krasznai and Gyory (1) who observed binucleated reticulum cells in the lymph nodes but stressed that typical Reed Sternberg cells were not present All side effects usually disappear when the drug is withdrawn However in recent years there have been several reports of the development of typical Hodgkin s disease and lymphosarcoma after anticonvulsant therapy with hydantoin derivatives For a recent review of the literature see Rausing and Trell (2) It is important that all such cases are reported

In the Cancer Clinic in Århus we have observed a patient in whom lymph node swelling increased after the withdrawal of diphenylhydantoin which had been given for epileptic seizures Biopsy of a lymph node was consistent with malignant lymphoma (lymphosarcoma)

I have had the opportunity to study the record of another patient who was treated with diphenylhydantoin for several years In this patient enlarged lymph nodes and a leukaemic blood picture developed Autopsy revealed enlarged lymph nodes along the aorta The histological findings were interpreted as malignant lymphoma (lymphosarcoma)

CASE REPORTS

Case 1

A divorced male worker aged 40 years was admitted to the Cancer Clinic in Århus in Jan 1974 with a diagnosis of malignant lymphoma and symptomatic epilepsy In 1965 he had sustained a traumatic lesion to the head A few years later typical epileptiform seizures developed and since Aug 1970 he had been treated in a neurological department with diphenylhydantoin (Phenytoin®) 100 mg in the morning and 200 mg in the evening The dose was increased to 200 mg twice daily in Oct 1973 Enlarged lymph nodes in the submandibular region were observed in Nov 1973 Phenytoin® was withdrawn and Tegretol® (a non hydantoin compound) treatment was instituted 100 mg twice daily increasing to 200 mg three times daily Instead of disappearing the swelling of the lymph nodes increased steadily and a biopsy specimen showed malignant lymphoma (lymphosarcoma)

He was transferred to the Cancer Clinic in Jan 1974 where he was treated with local irradiation following which the lymph node enlargement subsided Metastases were not found When he was last seen at the Cancer Clinic in Jan 1975 no reappearance of the lymph node swelling was found

Case 2

A married male messenger aged 61 years was admitted to the Department of Neurology in Århus in Dec 1959 suffering from symptomatic epilepsy which had persisted for about 6 months He had sustained head injuries in 1939 He had been treated with diphenylhydantoin (Phenytoin®) 200 mg in the morning and 100 mg in the evening with good effect From April 1964 the Phenytoin® dose was increased to 200 mg twice daily Treatment with Phenytoin® continued up to the patient s death in 1971

In March 1965 he was admitted to another hospital (in Herning) suffering from acute myocardial infarction and later he was repeatedly admitted to Herning Hospital for cardial incompensation the last time in Dec 1971 On this admission his blood picture was interpreted as that of myeloblastic leukaemia The leucocyte count was 16 600/μl with a differential count of myeloblasts 1% promyelocytes 3% myelocytes 2%

1% stab forms 12% segmented neutrophils 43% eosinophils 3% basophils 2% lymphocytes 22% monocytes 11% The sternal marrow showed diffuse infiltration with immature cells which were presumed to be myeloblasts A tumour the size of a goose egg was found in the left supraclavicular region enlarged lymph nodes were present in both angular regions A biopsy specimen of the supraclavicular tumour showed leukaemoid infiltrations The patient died suddenly on Dec 5 1971

Autopsy revealed a large glandular conglomerate on the left side of the neck and enlarged lymph nodes along the aorta and in the porta hepatis Infiltrations in the left kidney and splenomegaly were present Histological examination of several nodes showed alterations which were interpreted as malignant lymphoma (lymphosarcoma) Here the differential diagnosis presented certain difficulties Probably it was a case of lymphosarcomatosis with infiltrations of the bone marrow but at all events it must have been a malignant disorder of the haemopoietic system

As already mentioned all cases of malignancies which develop after administration of hydantoin derivatives should be reported It is not known why lymph node swelling in some cases results in malignant lymphomata but it seems clear that such drugs should be withdrawn as soon as lymph node swellings occur

ACKNOWLEDGEMENT

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Thyroid Carcinoma after ^{131}I Treatment for Hyperthyroidism

Report of a Case

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ABSTRACT A case of anaplastic thyroid carcinoma is described, occurring in a 71 year-old woman, 8 ½ years after treatment with 8 mCi ^{131}I for hyperthyroidism

Thyroid carcinoma is a rare finding after ^{131}I treatment for hyperthyroidism (3 4) Although a causal relationship seems improbable we feel these cases should be reported in order to get an impression of the incidence with which they occur

CASE REPORT

A woman born in 1903 was first seen at the Out patient Department of Medicine of our hospital in March 1966 because of symptoms of hyperthyroidism In 1943 a nodule had been removed from the thyroid elsewhere which was described as a thyroid adenoma but not examined histologically In 1947 a hysterectomy had been performed She was being treated for mild hypertension with reserpine

There was an elastic nodule in the right lobe Hyperthyroidism was confirmed both clinically and by laboratory tests BMR +76% serum iodoamino acid concentration (IAA) (1) 0 86 μmol I/l (normal 0 29–0 58) 24-hour thyroid uptake of ^{131}I 70% On the scintigram most activity was seen in the right lobe coinciding with the palpable nodule but the central part of this area was less active A treatment dose of 8 mCi ^{131}I was given on April 14 1966 Three months later the patient was clinically euthyroid and the IAA was normal (0 42 μmol I/l) She was subsequently lost to follow up

The patient was seen again at the Out patient Department of Medicine on Nov 8 1974 because of increasing goitre since Sept 1974 She complained of difficulties with breathing and swallowing and had been hoarse for one week In addition she had several symptoms suggestive of hyperthyroidism agitation increased perspiration palpitations and weight loss but euthyroidism with possible thyroid carcinoma was considered more probable She did not appear to be very sick at this time Apart from the large diffuse very firm goitre the physical examination yielded no abnormalities When she was seen at the Division of Nuclear Medicine on Nov 18 the goitre (estimated weight 200 g) was not painful or tender There was a slight ptosis of the right superior palpebra Thyroid malignancy was now considered very probable Lymph nodes could not be felt in the neck possibly because of the large dimensions of the goitre Euthyroidism was confirmed by laboratory tests serum hormonal iodine concentration (1) 0 46 μmol/l (normal 0 24–0 48) 24-hour ^{131}I uptake 25% Scintiphotography after 2 mCi ^{99m}Tc pertechnetate showed very little uptake in the gland a repeat scan with ^{131}I was planned

Because of rapid deterioration the patient was admitted to the hospital on Nov 20 A clinical diagnosis of anaplastic thyroid carcinoma was made The growth rate had apparently been very fast as during the short period of observation a definite increase in size had been noticed and alarming symptoms had developed An inspiratory stridor shortness of breath and a clear-cut Horner s syndrome at the right side were the dominant signs X ray examination disclosed multiple lung metastases The scan with ^{131}I was not made because increasing difficulties with breathing necessitated intubation on the evening of admission A thyroid biopsy showed anaplastic carcinoma The patient died on Nov 27 Autopsy was not performed

PATHOLOGIC FINDINGS

Grossly the two biopsies were about 1 5×1×0 7 cm greyish white Microscopically there is a carcinoma invading the thyroid parenchyma The tumour shows extensive necrosis The tumour cells are forming solid fields and are large pleomorphic and mitoses are numerous In some areas the cells are spindle shaped resembling those of a fibrosarcoma (Fig 1*a*) Bizarre cells often giant size are easily found (Fig 1*b*) The tumour has the characteristic features of an anaplastic giant cell carcinoma (5)



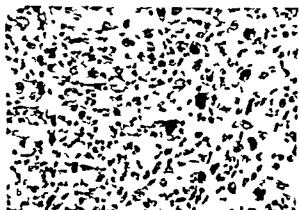


Fig 1 (*a*) Area simulating fibrosarcoma (×79) (*b*) F eld to illustrate the presence of giant cells (×317)

COMMENT

The incidence of thyroid carcinoma after treatment of hyperthyroidism with ³ I is very low McDougall (4) found only 15 documented incidental cases six of which occurred within five years of treatment In a systematic cooperative follow up study on 21 714 patients in the USA (3) only 19 cases were found which had been detected over one year after the (first) treatment dose when viewed in the light of the incidental malignant lesions found in patients primarily treated surgically (4 of 11 732 in addition to 50 cases occurring at the time of the operation or within one year of the intervention) the risk from ¹³¹I was not significant Children may form an exception in th s respect as four of the patients reviewed by McDougall (4) were under 20 years of age at the time of the ¹³¹I treatment whereas relatively very few children have been treated with ¹³ I Sheline et al (6) found thyroid nodules 5 10 years after ¹³ I treatment in 3 of 18 pat ents treated before the age of 20

Yet the possibility cannot be excluded that in the present case a (slowly growing) carcinoma was present already at the time of the ¹³ I treatment possibly in the hypoactive area Ded fferentiation of papillary carcinomas has occasionally been described and a causal relationship with irradiation has been suggested (2)

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Arterial Calcification and Progressive Peripheral Gangrene after Renal Transplantation

Report of Two Cases Treated with Parathyroidectomy

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ABSTRACT Two cases of progressive arterial calcification and peripheral gangrene after successful renal transplantation are presented Parathyroidectomy was performed and was followed in one case by healing of the peripheral necroses It is suggested that parathyroidectomy should be considered in similar cases even in the absence of hypercalcemia

Soft tissue and vascular calcifications are common in uremic patients particularly those with associated secondary hyperparathyroidism (10 13 17) After successful renal transplantation soft tissue calcifications have been reported to disappear (22) but vascular calcifications have with few exceptions been considered resistant Often arterial calcifications are present prior to maintenance haemodialysis and their incidence increases with time during such treatment (16 21) The arterial changes are of the Moenckeberg type of arteriosclerosis Usually they give no symptoms though they have been reported occasionally to cause peripheral gangrene (8 19) Similarly in some renal transplant recipients vascular calcifications have been found to progress causing peripheral gangrene in otherwise successfully treated patients (5 6 14 18)

This report concerns two cases with such development later after renal transplantation than has previously been reported Parathyroidectomy was performed which in one of the cases was followed by healing of the peripheral necroses

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CASE REPORTS

Case 1

A 51 year-old man in whom progressive pyelonephritis and severe hypertension was discovered in 1964 Family history revealed that he had a son with juvenile diabetes During the following three years his serum creatinine level increased from 3 5 to 18 mg/100 ml and he was referred to our hospital in 1967 for maintenance dialysis which was performed twice a week Towards the end of 1968 the serum calcium level was 5 0 mEq/l and serum phosphate 8 1 mg/100 ml Serum alkaline phosphatase levels were occasionally raised to 4 0–5 5 Bessey Lowry units During 1964–68 it was occasionally difficult to obtain a satisfactory regulation of BP Studies of serum lipids revealed a moderate hyperlipemia of the Fredrickson type IV

In Feb 1969 the patient obtained a cadaver renal transplant and his own kidneys were removed at the time of transplantation Conventional postoperative treatment with prednisolone and azathioprine was given Two rejection episodes occurred during the following two months and were successfully treated with a temporary increased steroid dose and with local irradiation of the transplant Since then renal function has remained stable Values at a 5 year check-up in March 1974 were serum creatinine 1 2 mg/100 ml inulin clearance 66 ml/min PAH clearance 264 ml/min and urinary protein excretion 0 2 g/24 h Since July 1970 the patient has received 5 0–7 5 mg prednisolone and 100–150 mg azathioprine per day

In Oct 1970 17 months after the transplantation the patient developed signs of peripheral ischemia in both legs While measuring the BP it was noted that the Korotkow sounds were almost impossible to hear Two months later an ulcer appeared over the left Achilles tendon which required a split thickness skin graft There was slow further progress of the peripheral ischemia with intensive pain in both feet even at rest In May 1972 gangrenous changes in the second third and fourth toes of the right foot became manifest Plain X ray of the extremities showed calcifications within large and small vessels including the digital arteries of both the hands and feet (Fig 1) Femoral arteriography showed occlusions of the



Fig 3 Case 2 Necrotic ulcerations of toes of the left foot 3 months before parathyroidectomy



Fig 4 Case 2 Massive calcification of the intima and media resulting in obstruction of a small artery of the amputated lower leg (×40)

but different degrees of development of calcification were encountered Early stages were characterized by small calcified garlands close to the internal elastic lamina or dots of calcification scattered throughout the media In more advanced lesions massive calcification of the media and elastic lamina was found Sections from the arteries of the amputated leg showed total occlusion of the lumen which was occupied by calcified tissue These morphological changes seem to bear a striking resemblance to those seen in diabetic macroangiopathy It has been shown (4) that a characteristic feature of the latter is a pronounced calcification of the medial coat giving considerable functional impairment

The pathogenesis of vascular calcification in uremia is probably multifactorial Derangement of calcium and phosphorus metabolism has been considered a major factor in the development of calcium deposition in soft tissues and blood vessels High serum levels of parathyroid hormone and signs of secondary hyperparathyroidism have been considered to be due to retention of phosphorus (3) and a disturbed metabolism of vitamin D (7) After renal transplantation normalization often occurs but some patients have constantly raised levels of parathormone for years in the absence of hypercalcemia (11 15) A high calcium phosphorus product in the blood is however frequently found in uremic patients The risk for metastatic calcifications is thought to be considerable when this value exceeds 70 but soft tissue and vascular calcifications have also been reported in cases with no elevation of this product (6 10 16) Though some regression of radiographically demonstrated arterial calcifications has been occasionally reported (14) vascular calcifications in uremia are usually much more resistant to the lowering of phosphorus levels in blood renal transplantation or parathyroidectomy than are periarticular or skin calcifications (10) One possible explanation might be harder binding of calcium to the arterial connective tissue than to other types of connective tissues Calcium deposits in arteries are probably preceded by changes in the ground substance This has been demonstrated in experimental studies (12) Some factors including hypercalcemia excess of parathyroid hormone and steroid as well as hypertension have been thought responsible for an accelerated change in the ground substance (16) A Moenckeberg type of arteriosclerosis particularly in the arteries of the legs has been reported in patients with rheumatoid arthritis after long term corticosteroid therapy (9)

In the reports of arterial calcifications with gangrene in uremic patients on dialysis (8 19) an elevated level of ionized calcium or a considerably

raised serum phosphate level appears to have been present Regarding the relatively rare progress of vascular calcification after renal transplantation it has been suggested (5 6 14 18) that the possible mechanism may be calciphylaxis (20) High levels of parathyroid hormone might then act as a sensitizing factor and cortison as a releasing factor This theory might be consistent with the absence of elevated calcium-phosphorus products in our two cases and in those described by Conn et al (6) and Coburn et al (5) whereas in other reports (14 18) at least elevated serum calcium levels have been noted

It seems difficult to evaluate the possible role played by the parathyroid glands in patients developing a posttransplant ischemic syndrome Tissue necroses have been reported to regress after parathyroidectomy (5 14) as happened in our first case In this patient the removal of apparently normal parathyroid glands appeared to favour the healing of progressive gangrenous lesions However in other cases treated with parathyroidectomy a progress has been reported leading to amputation and ultimate death (6 18) as in our second case

The indication for parathyroid surgery after renal transplantation is a matter of controversy However in patients whose prognosis seems to be very bad attempts to change the outcome appear warranted Until more knowledge of the posttransplant ischemic syndrome has been gained we suggest that subtotal parathyroidectomy should be tried at an early stage of this disease

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levels In extended studies some groups have felt that serial CEA determinations failed to be of clinical value while others have found a response in CEA levels corresponding to clinical improvement

Pancreatic adenocarcinomas have high contents of CEA High blood CEA values are common and the diagnostic accuracy higher than with any of the ordinary clinical methods of investigation alone Pancreatitis too gives a raised value in around half of the patients although usually not to levels exceeding 10 ng/ml A short report on a fetal antigen occurring in pancreatic adenocarcinomas only was recently published by Banwo et al Rabbit antiserum produced against fetal pancreas reacted with fetal pancreas and pancreatic carcinomas after absorption with normal pancreas Comparatively high levels of this antigen must be present in serum since it was detectable by immunodiffusion

Gastric cancer contains CEA and often gives raised serum CEA levels FSA is a fetal sulphoglycoprotein antigen which has also been associated with gastric carcinomas It is present in the fetal gut It cannot be detected in gastric juice of adults but was found in over 90 % of persons with gastric carcinomas (5) It has already been evaluated as a screening method for healthy persons in ages of increased risk and found to reveal previously unknown cancers In a large population study of 14 000 persons FSA was found in around 1 % Four persons had a clinically symptomless gastric cancer and one had gastric polyposis FSA is related to blood group antigens It has been speculated that the blood group specificity disappears during carcinogenesis while FSA specificity appears Possible detection of this substance in the serum has been hampered by the lack of a radioimmunoassay Deutsch et al described a tumor associated antigen with β mobility (GCA) in gastric cancer secretions which was not found in the tumor tissue The relation of this antigen to fetal tissues was however not established

Bladder tumors CEA values are increased in the urine patients with transitional cell carcinomas Serum CEA ls in patients with bladder tumors are increased in % of the patients in stages T3 and T4 and with metastasizing bladder tumors the mean serum level is further increased This means that simultaneous determination of serum and urinary CEA should be of value in the investigation of primary bladder cancer and at suspected recurrence or metastasis

An interesting substance from Wilm s *renal tumor* was described by Beierle et al The substance is a mucopolysaccharide present in the tumor blood and urine of these patients It does not occur in the normal adult kidney but cross reacts with calf fetuin

Hepatomas have a range of proteins also found in the fetal liver and with hepatic dysfunction in adults AFP is normally synthesized by a hepatic precursor cell It has been demonstrated late in human fetuses in tumors of hepatocellular origin and in teratocarcinomas (1) It may be found in very high levels in serum of patients with liver cancer Analogous proteins are found in fetal life in all investigated species having a more or less close immunological relationship to the human AFP α_2 H globulin or ferroprotein described by Buffe et al occurs in the fetal liver and serum Children with hepatitis jaundice hepatoblastomas and cholangiomas had rising serum levels with progressive disease A fetal protein with β mobility β fetoprotein (BFP) was also described by Alpert et al in fetal liver and hepatomas The protein is immunologically indistinguishable from normal liver ferritin It is increased in sera of adults with a variety of benign and malignant conditions No counterpart has been described in animals

AFP CEA or BFP were found in different percentages of malignant *teratoma* cells (9) In most cases there were also high or raised serum levels During tumor progression AFP and BFP occurred either alone or in combination with CEA and each other The prompt decrease in serum AFP and BFP after successful therapy and the low levels in serum of healthy males support the idea that tumor cells were responsible for the main part of the synthesis It is also likely that the proteins were synthesized by different cells and that functionally different subpopulations exist

Ovarian tumors may give raised serum CEA levels and the content of CEA or a CEA like substance is especially high in mucinous cystadenocarcinomas as described by Goldenberg et al Miroff et al described a β_1 globulin in ovarian tumors mainly occurring in the mucous fluid and of a lower molecular weight

Several antigenic systems have been described for *leukemias and lymphomas* and some of them were related to antigens occurring *in fetal life* The F antigen of Hodgkin s disease has been found in fetal liver and spleen and is thought to represent products of reactive lymphocytes while the S antigen found in early fetal liver and neonatal thymus is thought to represent a dedifferentiation antigen (7) Cells of chronic lymphatic leukemias have surface antigens in common with lymphoid cells of normal newborns A study by Lewis et al demonstrated another fetal antigen in the bone marrow cells of normal patients and increased in malignancies—notably in leukemias Mature polymorphonuclear cells did not have this latter antigen

A cytoplasmic antigen specific to human *sarcomas* has been described by Morton et al A similarly distributed antigen S_2 was found by Mukherji and Hirshaut in sarcoma cells benign giant cell tumors and in embryonic tissue of 8–12 weeks gestation Anti S_2 antibody was demonstrable by immunofluorescence in a high frequency in patients with sarcomas but also with other tumors

Avis and Morton have found reactivity to embryonic cells after immunizing rabbits with extracts of a *melanoma* Similarly extracts from human fetuses of 12–16 weeks gave antibodies with a reactivity to melanomas teratomas and colonic tumors but not to normal adult tissues

A fetoprotein with γ mobility GFP was extracted with saline by Edynak et al from a variety of human tumors both malignant and benign It was found in the human fetus as well as in bovine and feline fetuses This antigen may represent rather non-differentiated tissues since its range is quite broad

Regans alkaline phosphatase was originally identified by Fishman in lung cancer and has since been found in serum and tumors of patients with various malignant tumors It is indistinguishable from placental alkaline phosphatase and as such is normally present in the ma

ternal rather than the fetal circulation It is not present in non pregnant women A raised serum level is found in 4% of patients with different tumors

Biological properties of some fetal substances

The presence of a fetal equivalent in tumors has stimulated several hypotheses about their functions

Differentiation marker CEA is thought to represent a certain developmental stage of entodermally derived cells The cellular content increases with a higher degree of differentiation Malignant teratomas were considered interesting in that they represent different stages of maturation of embryo-like components which might also differ in synthesis of fetal antigens Several fetal antigens were found in such tissue but were not seen in the same cells The mechanism by which the expression in a tumor is provoked is speculative (2) The phenomenon may however be of importance for indicating prognosis Similarly other fetal proteins many of which reappear in tumors may be characteristic for a certain stage of neonatal development

Exchange protein Another glycoprotein gradually follows CEA in the colonic mucosa during the latter part of fetal life It has partial immunological cross reactivity and a lower molecular weight Similarly FSA cross-reacts with two glycoproteins in normal gastric juice one of gastric and one of intestinal origin Blood group activity has been described both for CEA preparations and FSA The blood group sites do not participate in the CEA–anti CEA reactions

Carrier proteins Apoferritin is an iron binding protein It is not known if the carcino-fetal ferritin-like protein BFP has the same properties AFP has been described to have affinity for oestrogens and may together with albumin act as an oestrogen carrier

Enzyme activity Esterase activity has previously been described in human colonic cancer tissue and in other malignancies such as hepatoma of rat Appreciable amounts of esterase activity have been described in 30% of colonic cancer but it was absent in a number of other malignant tissues Enzymatic activity similar to that of esterases was recently described by Munjal and Zamcheck for purified CEA The CEA preparations gave hydrolysis of chromogenic substrates with acyl groups

Immunogenicity Immunological identity reactions between antigens found in fetal life and in tumors have been demonstrated for several substances Therefore tolerance to these self antigens has generally been assumed This is not the case with all fetal antigens in animals and has lately also been questioned in humans Antibodies against CEA were described by Gold et al in some patients with colonic cancer whereas Collatz et al could not find any evidence for autoimmunity to CEA The search for cell mediated immunity has not shown any evidence for reactivity to purified CEA in humans Due to the above reasons antibodies to fetal antigens have been produced in animals for diagnostic purposes

Cell growth regulating properties Embryonic antigens have recently been proposed to have immunoregulatory functions (8) Cell mediated specific or non specific activities have been demonstrated in experimental systems

It is less likely that fetal antigens would inhibit immunity specifically in humans since immunological tolerance is believed to be the rule at least for AFP and CEA However enhanced tumor growth due to inaccessibility of other growth-regulating substances may be expected if antigen synthesis is high (9)

Clinical usefulness

Valuable information as an aid in diagnosis of tumors may be obtained by studying fetal antigens in the tumor tissue or antigens released into the blood

The tissue content of a certain antigen may be correlated with the degree of differentiation of the tumor cells It is hoped that further combined studies of several fetal proteins will indicate the degree of differentiation or stage of antigen expression which an individual tumor represents It should be of value to determine if subpopulations of cells each with differing antigen synthesis exist in one tumor Perhaps these antigens can then act as markers for the cellular differentiation and thus also for the selection of type of therapy

The release of fetal antigens to blood is of practical importance The demonstration of high CEA AFP and/or BFP in serum appears to be an indicator of poor prognosis

Extensive studies have confirmed that CEA in blood is increased in around 75% of entodermally derived carcinomas but also in 40–50% of non-entodermal cancer The extent of the rise is usually parallel to the clinical stage of the tumor Although the highest values are encountered in cases with metastases or advanced tumors rises are seen also early at recurrence and during follow up postoperatively or post irradiation This justifies blood CEA determinations being performed at the clinic A decreased blood CEA can be expected with effective therapy Persisting or increasing values indicate resistance to therapy or recurrence of a tumor

A limitation to clinical application is the fact that a raised CEA is frequently encountered in chronic infections or inflammatory diseases These values are usually borderline but may sometimes be impressive They decrease—like CEA levels of cured tumor patients—when the inflammatory state has subsided

AFP occurs in very high serum levels in most hepatocellular cancers and is often raised with malignant teratomas and gastric cancer BFP is raised with hepatomas and several other liver dysfunctions FSA level is high in gastric juice in the majority of patients with gastric cancer Several other fetal antigens are of potential interest as adjuvant marker proteins for diagnosis of various tumors

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Treatment of Sinoatrial Syndrome with Permanent Cardiac Pacing in 90 Patients

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ABSTRACT Out of a pacemaker population of 392 patients, 90 (23%) have been found to have sinoatrial syndrome Their ages ranged from 22 to 86 years, and averaged 66 for men and 70 for women The male to-female ratio was 1 1 6 Before pacemaker implantation, syncopal attacks had occurred in 54% dizziness without syncope in 31% and tachyarrhythmias in 57% Atrial or paroxysmal supraventricular tachycardia had been recorded in 33%, atrial fibrillation or flutter in 28%, and ventricular tachycardia in 11% First and/or second degree AV block was found in 36% Coronary heart disease was present in 61% and 20% had had myocardial infarction Cardiomyopathy and previous carditis were other associated heart diseases Sinoatrial syndrome was the only manifestation of heart disease in 20% Follow up time after pacemaker implantation ranged from 3 months to 7 years, mean 23 months Syncopal attacks were stopped in 48 of 49 patients, dizziness was relieved in all 28 patients and tachyarrhythmias were controlled by combined drug treatment in 43 of 51 patients Nineteen patients died during the follow up, most of them of cerebrovascular events or myocardial infarction Associated coronary heart disease was especially frequent in this group The death of one patient was caused by a run away pacemaker Other pacing failures were due to electrode movement or premature battery exhaustion There was no mortality associated with pacemaker implantations or replacements These results strongly support the view that pacemaker treatment most effectively controls symptoms of sinoatrial syndrome when drug treatment fails

The role of permanent pacing in the treatment of Adams Stokes syndrome due to atrioventricular block is well established During recent years however the number of pacemakers inserted into patients with sinoatrial rhythm disorders has been steadily increasing This is due to the fact that disturbances in sinus impulse formation or transmission to the atrial myocardium together with delay or lack of escape beats or escape rhythm give rise to bradycardia which usually does not respond to medical treatment In addition many patients with this type of bradycardia have bouts of paroxysmal tachycardia which renders medical treatment even more difficult Spells of dizziness and syncopal attacks occur due to sinus arrest sinoatrial block extremes of heart rate and asystole after abrupt cessation of tachycardia

This wide spectrum of rhythm disturbances is regarded as a syndrome which has been given several names like sick sinus syndrome (8 15) lazy sinus syndrome (12) bradycardia tachycardia syndrome (24) tachycardia bradycardia syndrome (13) or sinu atrial disorder (14) None of these names seems ideal and the term sinoatrial syndrome is used in this report on 90 patients with varying sinoatrial rhythm disorders treated with permanent pacing

MATERIAL AND METHODS

To identify sinoatrial syndrome as the cause of pacemaker treatment the records of 392 patients were reviewed in whom a permanent pacemaker had been inserted between Jan 1966 and March 1973 at the University Central Hospital Helsinki Patients with complete AV block were excluded as were patients with myocardial infarction occurring less than 3 months before pacemaker insertion Thus 90 patients were found with sinoatrial rhythm disturbances as the cause of pacemaker implantation The following ECG criteria were used for classification of sinus node dysfunction preceding pacemaker treatment

Sinus arrest was regarded as the cause of dropped beats if the ensuing PP distance differed by more than 0 1 sec from the multiple of normal PP intervals or if asystole

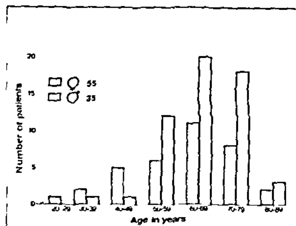


Fig 1 Distribution of patients with sinoatrial syndrome according to sex and age

was followed by an escape beat or escape rhythm The term sinus arrest includes also third degree sinoatrial block because they cannot be separated strictly electro cardiographically Two cases with atrial fibrillation and severe bradycardia present for several years were also classified as due to sinus arrest

A second degree *sinoatrial block* type II was diag nosed if a prolonged PP distance did not differ by more than 0 1 sec from the multiple of normal PP dis tances A sudden doubling of a slow sinus rhythm was considered to indicate type II second degree sinoatrial block with 2 1 conduction A second degree sinoatrial block type I (Wenckebach) was not found in this material

Sinus bradycardia was diagnosed if the patient s heart rate at rest was persistently less than 60 beats/min Bradycardia caused by medical treatment or unusual vagal e was excluded by omitting treatment and giving ine i v

records of the 90 patients who met the criteria were analysed with respect to clinical history occurrence and type of tachycardia medical treatment indications for pacemaker insertion and subsequent clinical course Most of the patients had been hospitalized many times and given different medical treatments before the decision was made to start pacemaker treatment Follow-up informa tion was obtained from the records of the pacemaker clinic where patients were seen one month after hospital discharge and at intervals of three months thereafter (10) If the patient had been referred to another hospital follow up information was obtained from records kept there

RESULTS

Age and sex distribution of the 90 patients (55 wo men, 35 men) is shown in Fig 1 Their ages ranged from 22 to 86 years The mean age at pacemaker insertion was 70 years for females and 66 years for males

In the course of the disease most patients had complained of several *clinical symptoms* caused by disturbance of heart rhythm 49 (54%) had experi enced one or several syncopal attacks Most of them had also had spells of dizziness Dizziness without syncope had occurred in 28 patients (31%) Seventeen patients had had bouts of tachycardia causing palpitation and anginal pain Since half of the patients with syncope or dizziness had also had attacks of tachycardia paroxysmal tachycardia was actually recorded in 51 patients (57%) In two pa tients with severe congestive heart disease brady cardia made digitalization impossible There was only one patient in this material without clinical symptoms in spite of sinus bradycardia and sino atrial block His ECG showed also left anterior hemiblock and pacemaker treatment had been con sidered justified as a preventive measure

The *duration of symptoms* varied from 2 months to 22 years mean 2 9 years More than 2/3 of the pa tients had had symptoms for more than a year be fore pacemaker implantation

Disturbances of rhythm and conduction found in ECG tracings are summarized in Table I Twenty five patients (28%) had a PQ time exceeding 0 20 sec The occurrence of different types of sinus node dysfunction are shown in Fig 2 Tachyarrhythmias and disturbances of AV conduction occurred in about the same percental distribution in the sub-groups of Fig 2

Table I *Rhythm and conduction disorders in 90 patients with sinoatrial syndrome*

Many patients had more than one rhythm disorder

	N	%
Paroxysmal supraventricular and/or atrial tachycardia	30	33
Atrial fibrillation or flutter	25	28
Ventricular tachycardia	10	11
1st and/or 2nd degree AV block	32	36

Table II *Associated heart diseases in patients with sinoatrial syndrome*

	N	%
Coronary heart disease	55	61
Cardiomyopathy	7	8
Previous carditis	9	10
Seclusion of atrial septal defect	1	1
None	18	20

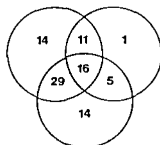


Fig 2 Occurrence of sinus bradycardia sinoatrial block and sinus arrest in 90 patients with sinoatrial syndrome

The *associated cardiac diseases* are shown in Table II Coronary heart disease was based on a history of angina pectoris or myocardial infarction or ECG evidence and was the most common diagnosis Eighteen patients (20%) had sustained myocardial infarction In six of the nine patients with previous myocarditis the diagnosis was based on hospital records and ECG findings during the acute phase In three patients the diagnosis was less certain Diphtheria was the cause of myocarditis in five patients Six patients had cardiomyopathy of the congestive type All these patients had congestive heart disease of unknown origin without any evidence of coronary heart disease One patient had hypertrophic cardiomyopathy In 18 patients (20%) sinoatrial syndrome was the only definite manifestation of heart disease Nine patients in this group had *other associated diseases* like diabetes hypertension and history of thyrotoxicosis each diagnosis occurring in three patients respectively In the whole material diabetes essential hypertension and previous thyrotoxicosis occurred in nine five and seven patients There were two siblings brother and sister who possibly had a familiar type of sinoatrial syndrome One of them had had diphtheria and is included in the group with previous myocarditis

Treatment

Before pacemaker insertion pharmacologic treatment with drugs speeding heart rate and/or with antiarrhythmics had been tried in most cases (Table III) In patients who are listed as having been treated with digitalis only pacemaker treatment

Table III *Drug therapy before and after pacemaker implantation in patients with sinoatrial syndrome*

	Before	After
Digitalis	11	41
Digitalis+antiarrhythmic drugs	18	25
Digitalis+antiarrhythmic +sympatomimetic+anticholinergic drugs	19	0
Digitalis+sympatomimetic +anticholinergic drugs	22	0
Sympatomimetic+anticholinergic drugs	18	0
Antiarrhythmic drugs	2	13
No drugs	0	11

Table IV *Main clinical indication for pacemaker insertion*

	N	%
Syncope	49	54
Dizziness	21	23
Treatment of tachyarrhythmias	17	19
Congestive heart failure	2	2
Preventive measure	1	1

was started after omitting digitalis and trying i v atropine or single doses of sympathomimetic drugs The predominant clinical indications for pacemaker insertion as stated in the records are given in Table IV Episodes of syncope and dizziness was the main indication in 77% of cases It should be pointed out however that about one half of these patients had tachyarrhythmias as well In 17 patients in whom treatment of arrhythmias was considered the main cause of pacemaker insertion bouts of tachycardia could not be controlled with digitalis and/or antidysrhythmic drugs because of bradycardia and conduction disturbances

Insertion of the endocardial electrode and the subcutaneous pacemaker was done in two stages After positioning of an Elema 588 unipolar electrode into the apex of the right ventricle external pacing was used for several days to detect early pacing failures Endocardial pacing was accomplished in 67 patients (75%) Myocardial electrodes were used in 23 patients (25%) in some because of young age in others because of an increase in the threshold or repeated movement of the electrode tip In nine patients fixed rate pacemakers were implanted initially between 1966 and 1968 They were later replaced by ventricular inhibited or ven

Table V *Follow up times (months) of 90 pacemaker treated patients*

	N	%
3–6	23	26
7–12	16	18
13–24	22	25
25–36	12	13
37–48	10	11
>48	7	7

tricular triggered (demand) units In all other patients demand pacemakers were used

Follow up and effect of pacemaker treatment

The follow-up time ranged from 3 months to 7 years mean 23 months giving a total of 172 patient years of pacing Fifty-one patients (57%) were followed up for more than a year (Table V)

Of the patients with syncope one only continued to have similar attacks as before probably due to inadequate cerebral circulation Three patients in this group had attacks of tachycardia but without syncope Dizziness was relieved in all cases Of the 17 patients with tachyarrhythmias causing palpitation and anginal pain 5 continued to have tachyarrhythmias in spite of combined pacemaker and medical treatment Effective treatment of congestive heart disease with digitalis became possible in th cases with this indication

Thus symptoms due to sinoatrial syndrome ther disappeared or were considerably relieved by pacemaker treatment in 83 of 89 patients This success is also reflected in changes in the pharmacological treatment (Table III) In two patients one with syncope and one with congestive heart disease as the main complaint permanent atrial fibrillation with a proper heart rate appeared after 6 and 10 months of pacing which made further pacemaker treatment unnecessary

Pacing complications

During the follow up period up to July 1973 a total of 160 pacemakers had been implanted into these 90 patients Sixty two pacemakers were replaced because of a change in the stimulation rate or impulse duration or other generator failures 26 of them within less than one year In 7 of these cases an emergency replacement was undertaken four times because of a run away pacemaker and three times because of sudden pacing failure In all other cases the replacement was done electively Two pacemakers belonging to a faulty series were replaced 2 months after implantation on request of the producer

In addition 6 pacemakers were replaced because of infection or local reaction four of them in one patient Bacterial infection or a local reaction of the implantation site occurred in a further 9 patients In 4 of them the pacemaker was resited in 5 the reaction subsided during conservative treatment

Because of movement of the catheter tip rise in the pacing threshold a broken wire or diaphragmatic stimulation endocardial electrodes were replaced during the follow-up in 18 of 67 patients Three of the 23 myocardial electrodes were replaced for similar reasons

Mortality

Nineteen patients (11 women 8 men) (21%) died between one month and 4 1/2 years after the initial implantation Their mean age was 68 years (range 37–85) and 2/3 of them were over 70 Based on the 172 patient years of pacing the annual mortality was 11% The causes of death as listed on the official death certificates are shown in Table VI Autopsy was performed in 6 patients in the others the cause of death had been considered certain on account of valid clinical data The death of one patient was apparently due to sudden pacing failure This woman of 59 years became suddenly unconscious and was taken immediately to the nearest hospital At her arrival the pacemaker was found to be firing at the rate of 360/min Resuscitation of the patient was unsuccessful One patient aged 37 with sinoatrial syndrome after myocarditis committed suicide in a mood of depression Excluding these two patients 13 of the remaining 17 patients who died had coexisting coronary heart disease and 6 had previous myocardial infarction In 4 patients no other heart disease than dysfunction of the sinus

Table VI *Causes of death after pacemaker implantation*

	N
Cerebral embolism or thrombosis	9
Myocardial infarction	7
Congestive heart failure	1
Suicide	1
Pacing complication (run away)	1
Total	19

node was diagnosed however two of them had diabetes mellitus one had cerebral atherosclerosis and one had previously had thyrotoxicosis Two patients who had also carcinoma died from cerebrovascular events As a group the patients who died did not differ from the whole patient material with regard to the type of sinus dysfunction occurrence of tachyarrhythmias or of first or second degree AV block

DISCUSSION

In recent years the number of pacemaker implantations because of sinoatrial rhythm disturbances has been steadily increasing Earlier reports on pacemaker treatment contain relatively few cases of sinoatrial disease (1 2) In 1971 Rasmussen (19) reported second degree sinoatrial block in 10% of pacemaker treated patients from Norway Conde et al (4) found 31 patients with bradycardia tachycardia syndrome among about 500 patients treated with permanent ventricular pacing In a recent publication from England (26) corresponding figures were 20 of 372 In a report from Denmark on 170 patients with Adams Stokes syndrome sino-atrial block was the cause of syncope in 39 patients or 23% (25) In a prospective study from Norway (22) the incidence of chronic sinoatrial disease was 51 against 98 patients with advanced AV block or 34% Of the patients with sinoatrial disease 23 needed pacemaker treatment which gives an incidence of 24% of all pacemaker treated patients The present material of 90 patients with sinoatrial syndrome was selected from a total of 392 patients treated by permanent pacing which gives a relative incidence of 23% Thus at least in Scandinavia pacemaker treatment has been started lately in one of four patients because of sinoatrial syndrome

This increment of pacemaker implantations because of sinoatrial syndrome has several reasons More attention has been paid lately to sinoatrial rhythm disturbances and to serious symptoms caused by sinus bradycardia sinoatrial block or tachyarrhythmias alternating with bradycardia (7 8 9 19 23 27) Bradycardia and sinus arrest may give rise to cerebral symptoms like syncopal attacks and spells of dizziness which are very disabling and may be even hazardous Many patients with sinoatrial syndrome suffer from palpitation and anginal pain during bouts of tachycardia and upon its abrupt cessation prolonged asystole may occur causing Adams Stokes seizures This is due to the inability of the sinus node to assume its pacemaker role after first ectopic rhythms In some patients bradycardia inhibits efficient treatment of congestive heart failure Only some of the patients with sinoatrial syndrome stay completely free from symptoms

Different opinions exist about the treatment of asymptomatic patients with sinoatrial syndrome Although pacemaker treatment has been suggested for all patients with the syndrome because of its potential hazards (9) common practice seems to be to withhold specific therapy in the asymptomatic patient (16 23) This has been our policy too Even in patients with minor symptoms medical treatment is usually tried first Actually in some patients bradycardia responds favourably to drugs speeding the heart rate without an increase in ectopic activity (11 22)

In some patients it is possible to control tachyarrhythmias by careful titration of antiarrhythmics There are also patients who have a transient disturbance of the sinus node function e g during acute myocardial infarction (21) and those in whom symptoms are precipitated by digitalis or other drugs Some patients having alternating sinus bradycardia and atrial fibrillation with a fast heart rate can be treated adequately with digitalis and β blocking agents as soon as sinus rhythm no longer reoccurs

However medical treatment proves successful only in some of the patients with sinoatrial syndrome In the prospective study of Rockseth and Hatle (22) about one half of the patients with sinoatrial disease needed pacemaker treatment Failure to obtain acceptable results with medical treatment has been emphasized in many publications on pacemaker treatment of sinoatrial syndrome (23 25 27) For most cases with sinoatrial syndrome permanent pacing with a demand pacemaker offers the most effective way of treatment and this is the main reason for the increasing number of pacemaker implantations In the present material syncopal attacks were stopped by pacemaker insertion in 48 of 49 patients Near syncopal attacks or dizzy spells disappeared in all 28 patients with such complaints Sigurd et al (25) obtained the abolition of cerebral attacks in all of their 39 patients treated with pacing In another study (3) syncopal or near syncopal attacks were st

32 of 35 patients Similar excellent results on cerebral symptoms have been reported by many others (4 6 22 23)

The use of an artificial pacemaker alone prevents the reoccurrence of tachyarrhythmias only in a small number of patients (23 25) In the present series the combination of appropriate antiarrhythmic agents with implanted pacemakers provided control of arrhythmias in 43 of 51 patients a success rate similar to that obtained by others (4 22 25) Some patients will continue to have tachyarrhythmias after pacemaker implantation in spite of maximal drug treatment (25) however cerebral symptoms due to serious bradycardia or asystole will be avoided

If bradycardia inhibits the use of digitalis in congestive heart failure and diuretics and sympatomimetics are not effective pacemaker treatment may be tried There were two patients in this material with this indication Pacemaker treatment proved successful in both of them Alleviation of symptoms of congestive heart disease has also been mentioned by other authors (3 22)

Pacemaker treatment is not free from complications The death of one patient in this series was probably caused by a sudden rise in the pacing frequency Twenty six units had to be replaced within less than a year because of premature battery exhaustion Lately the maximum life span of cemakers has increased to about 3 years and n away failure has become extremely rare making pacemaker treatment easier and safer There were no deaths associated with pacemaker implantations or replacements in this material Electrode complications occurred in 21 patients Main causes were movement of the catheter tip exit block due to increased threshold or a broken wire This type of complications will continue to occur in spite of technical improvements to pacemakers Myocardial electrodes proved to be more reliable than endocardial ones however since the patient with sinoatrial syndrome is not completely dependent on artificial pacing the pervenous approach is more recommendable

Although atrial pacing has some theoretical advantages in sinoatrial syndrome as long as AV conduction is intact ventricular pacing was used in this material as usual Recently permanent pervenous atrial pacing with the electrode positioned in the coronary vein has proved to be a safe and reliable technique (16 20) Atrial pacing may prevent the occurrence of atrial tachyarrhythmias in some cases but if antiarrhythmic drugs have to be used, ventricular pacing is more reliable because of possible further impairment of AV conduction by drug therapy

The aetiology of sinoatrial syndrome is not clear The most common associated heart diseases are coronary heart disease previous carditis and cardiomyopathy In one series (19) 6 of 21 patients had had diphtheria In another study 10 patients (20%) had previously had rheumatic fever (22) Both diagnoses occurred in 11% of 46 patients with unexplained sinus bradycardia (7) but were less frequent in the present material as in many others (3 23 25) At younger ages the diagnoses of carditis and cardiomyopathy prevail and seem to be an aetiological factor of sinoatrial syndrome however regarding the whole problem they play a relatively minor role as do congenital heart diseases

In the present series the majority of patients (61%) had coexistent coronary heart disease Coronary heart disease has been reported in patients with sinoatrial syndrome in 28% (25) 36% (23) about 50% (4 16) and 73% (3) Its aetiological importance for the occurrence of the syndrome is controversial because about 1/3–1/5 of patients have sinoatrial syndrome as the only manifestation of heart disease Also the male to-female ratio of about 1 1 in patients with an average age of 63 has been used as an argument against coronary heart disease as a dominant aetiological factor (25) This applies even more so if the male to-female ratio is 1 1 6 as in the present study Davies and Pomerance (5) have shown that appreciable muscle fiber loss occurs in the sinoatrial node in clinically normal people with age starting around 60 years It is probable that such degenerative changes are speeded up by vascular disease A concept of primary changes in the sinoatrial node and conducting tissue adversely affected by vascular changes of the sinus artery and other coronary arteries offers an explanation of many features of sinoatrial syndrome especially in elderly patients The relatively good prognosis in patients with sinoatrial syndrome as documented by the long duration of symptoms in many patients has been pointed out by several authors (7 23) It seems however that there is an excess of cerebral embolism especially in patients with paroxysmal tachycardia (6 18 23) In most patients with the syndrome coexisting heart disease will influence the sur l as exem-

plified in this study by the high incidence of coronary heart disease and previous myocardial infarction in those patients who died during the follow up

The electrophysiologic basis of sinoatrial syndrome seems to be a universal slow down or lack of spontaneous depolarization of the sinus node as well as of other conducting tissues This explains the occurrence of extreme bradycardia and the lack of escape beats and escape rhythms ECG analysis of the present material according to the type of sinus node dysfunction (Fig 2) did not reveal any differences between subgroups with regard to tachyarrhythmias or AV conduction disturbances First and second degree AV block recorded in 36% of the present patients seems to be quite common in sinoatrial syndrome Atrioventricular and interventricular conduction disturbances have been reported in 20% (25) to 60% (17) Not a single case of sinoatrial syndrome proceeding to third degree AV block was found in this material Apparently little overlapping occurs between sinoatrial syndrome and total AV block as noticed by Sigurd et al (25)

At first sight the occurrence of tachyarrhythmias in a heart which lacks spontaneous depolarization and escape beats seems contradictory However bradycardia favours the occurrence of ectopic activity and the resulting depolarization wave finds the stage set for re-entry and circulus movement Actually most of the tachyarrhythmias in sinoatrial syndrome are of the circulus movement type On sudden cessation of the tachycardia a prolonged asystole usually occurs in these patients because of the very strong slow-down effect of overdrive pacing on spontaneous depolarization in sinoatrial syndrome Consequently the so-called bradycardia tachycardia syndrome is an important aspect of sinoatrial syndrome

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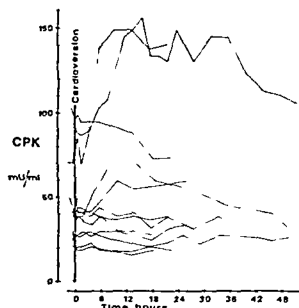


Fig 1 Serum CPK curves following cardioversion

diately after the DC shock was 14 mU/ml (29%) The maximal CPK rise during the 18–51 hour period was 78 mU/ml (110%) In no case did the CPK exceed 156 mU/ml during the period of investigation The CPK rise showed no correlation with either the discharged energy or the number of DC shocks

DISCUSSION

…ioversion of supraventricular and ventricular tachyarrhythmias is a routine method for treating these complications in AMI If cardioversion influences the serum CPK activity e g by release of CPK from thoracic muscles this procedure impairs the diagnosis of AMI by CPK estimations

Increased serum CPK values above the upper normal level have in fact been reported after cardioversion (5 8 11 30) Konttinen et al (11) found a CPK increase in 8 of 37 patients The CPK rose 1 2–29 times the normal level and the maximal increase occurred one day after the DC shock The patients were anaesthetized with sodium thiopental 0 5 mg of atropine being used as premedication It cannot be concluded from this report or from any of the others (5 8 30) whether i m injections had been given before or during the investigation period I m injections have been reported to induce elevations of serum CPK with a maximum 24 hours after the injection (2 10 15 17 28 31)

In the study by Konttinen et al (11) blood samples for CPK determination were drawn 3 hours before the shock and on 3 successive mornings afterwards The maximal CPK increase occurred in the morning after the DC shock In our study there was no tendency to elevation of the CPK curves towards the end of the period Even if an increase does occur after the first 24 hours it will not interfere with diagnosing AMI since the CPK peak is reached 12–24 hours after the onset of symptoms (19 25)

In the present series of 12 patients only two had a CPK rise above the normal level which in our experience is considered to be 130 mU/ml This incidence does not differ from the corresponding figures 8 of 37 patients reported by Konttinen et al (11) The great difference is that our maximal CPK increase of 110% was much lower than their lowest of 450% Using our techniques of cardioversion and CPK analysis a diagnostic dilemma will very seldom occur as both a slightly abnormal CPK maximum and especially a DC shock during the first hours after onset of symptoms are uncommon in AMI A DC shock after the initial phase of AMI may in a minority of patients give a minimal CPK rise which however would cause diagnostic difficulties only in patients with recurring ischemic pains

Explanations of the different results in the present study compared with others (5 8 11 30) may be different cardioversion procedures including methods for anaesthesia and sites of injections and differences in the frequency of blood sampling as well as in methods of CPK analysis These possibilities cannot be further evaluated because of lack of information in these reports

It has previously been discussed whether the enzyme elevations observed after cardioversion are caused by heart muscle damage or skeletal muscle damage (because of contraction of the skeletal muscles caused by the DC shock) (11 30) Using CPK isoenzymes it has been shown that the elevation of total CPK in 15 of 40 electroconversions was of skeletal type (CPK MM) (14) There are however divergent opinions about the proportion of CPK MB in skeletal muscle 0–20% (3 26 29) as well as about the percentage of CPK MB in total CPK in association with AMI 0–38% (13 29) Therefore there is a certain risk of both false positive and false negative diagnosis of AMI even with the aid of CPK isoenzymes

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Deaths and Non-fatal Reinfarctions during Two Years' Follow-up after Myocardial Infarction

A Follow up Study of 440 Men and Women Discharged Alive from Hospital

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ABSTRACT Since 1968 special units for registering and following up all myocardial infarction patients have been in operation in Göteborg The present paper reports on the deaths and non fatal reinfarctions among 440 men and women below 67 years of age followed for two years after hospitalization for myocardial infarction Of the 359 men 299 had their first infarction and 60 a recurrent infarction During two years 13% of men with a first infarction and 37% of men with a recurrent infarction died Deaths were significantly more common during the first than during the second year There was no significant difference in mortality between men and women The incidence of non fatal reinfarctions was of similar magnitude and showed similar time relationships Two thirds of the deaths were sudden (within 24 hours after onset of new symptoms) and half of the deaths occurred outside hospital The autopsy rate was 92% Two thirds of the deaths were caused by a fresh morphological infarct In one fourth of the deaths where no cause was found at autopsy death was ascribed to malignant arrhythmias Heart weights were higher among patients dying after recurrent infarction at entry to the study No distinctive pattern was found when autopsy findings were correlated to the interval between the onset of symptoms and death Deaths due to fresh infarction and without an identifiable cause were more common among those who died within 24 hours of onset of symptoms compared to those dying after longer intervals

The hospital mortality from acute myocardial infarction (AMI) has attracted much interest (6 37 39 43 53) but there is also a considerable mortality during the years immediately following infarction (2 7 17 25 30 45 66)

The occurrence of reinfarction among patients discharged alive from hospital after myocardial infarction has been studied previously (8 14 35 53 59) These studies however do not always distinguish between fatal and non fatal reinfarctions Non fatal reinfarctions are of interest as they worsen the prognosis (29 40)

The present study reports the mortality causes of death autopsy findings and occurrence of non fatal reinfarctions during two years follow up in a non selected material of men and women below 67 years of age discharged alive from hospital after myocardial infarction in Goteborg

PATIENTS AND METHODS

The material comprised 440 patients 359 men and 81 women with myocardial infarction discharged alive from hospital during 1968–70 299 of the men and 65 of the women had their first infarction Patients who had suffered clinical infarction prior to entering the study were classified as having suffered reinfarction As from 1968 all cases of AMI occurring in certain age groups in Goteborg are registered in a special Myocardial Infarction Register (19 67) During 1968–69 patients below the age of 55 years were included in the study and in 1970 patients up to the age of 67 years The material comprised approximately 90% of all diagnosed surviving cases of myocardial infarction in the total population of Goteborg in the age groups concerned (19) All surviving patients were systematically cared for after discharge from hospital at a special Post myocardial Infarction Clinic (19 55 63) A random sample comprising 30% of the men aged 57–67 years was

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Degree of atherosclerosis in coronary arteries

No atherosclerosis 0
Single atherosclerotic plaques (<25% of surface) 1
Some degree of atherosclerosis (25-75% of surface) 2
Advanced atherosclerosis (75-100% of surface) 3
Mark on figure site of stenosis I
occlusion (no acute thrombosis) II
acute thrombosis III

Heart weight g



Condition of myocardium
Mark on figure by circumscription of the area the following conditions
Acute infarct
macroscopically certain A1
probable A2
suspected A3
Infarct in organization B
Scar of infarct (>1/2 cm²) C
Diffuse fibrosis D
Mural thrombosis E
Ventricular aneurysm F
Rupture G
Material from heart for microscopic investigation
Yes No



Fig 1 Post mortem findings Registration form

excluded in order to form a reference group for evaluation of this clinic (57) Some of the patients above 57 years of age were treated with β blockers and some below 57 participated in a programme of supervised physical training (49) Since exclusion of these patients did not have any marked influence on the results all patients have been included

All death certificates issued in Göteborg for the relevant age groups were examined continuously by the registration organization In addition to cause of death the death certificates also specified the basis on which the cause of death was established e g clinical autopsy forensic autopsy clinical diagnosis etc Patients who were in hospital at the time of death were generally submitted to clinical autopsy and most of those who died outside hospital were autopsied by a forensic pathologist after a police enquiry Thus autopsies were performed by several different people In relevant cases forensic chemical analyses were performed

Uniform collection of autopsy data was achieved by utilizing a special form developed in collaboration with pathologists from the Institute of Pathology which was filled in by the person performing the autopsy at the time of autopsy (Fig 1) (19) The form included data on heart weight extent of arteriosclerosis of the coronary vessles (as a percentage of the total surface) stenoses and occlusions both fresh and older Any myocardial fibrosis healed infarction scars and acute infarction changes were also registered The macroscopic state of the coronary vessels was assessed visually while in some cases the myocardium was investigated microscopically

For myocardial infarction to be recorded as the cause of death in the autopsied patients morphological evidence of fresh infarction was required For non-autopsied patients the clinical criteria were the same as for the diagnosis myocardial infarction at entry to the study The diagnosis required two of three criteria to be fulfilled (19)

The deaths in the total material in which no extracardiac or cardiac cause of death could be found were ascribed to acute arrhythmias They have been included in the total cardiovascular group

Deaths occurring outside hospital were subjected to routine police enquiry by special police personnel These enquiries concerned the circumstances of death such as where death occurred as well as the interval between onset of any symptoms and death Witnesses were questioned concerning symptoms In addition relatives of the deceased were interviewed by staff from the Post myocardial Infarction Clinic

Information on non fatal reinfarctions was obtained via the Infarction Register Hospital case records were studied and the diagnostic criteria were checked In three cases of reinfarction not leading to admission to hospital the diagnosis was based on the history of characteristic pain and ECG recordings when the patient attended for a previously arranged check-up Non-fatal infarction was considered to have occurred when two of the three criteria—pain ECG changes and transaminase changes—were fulfilled i e the same criteria as for entry to the study Reinfarction was defined as non fatal when the patient was discharged alive from hospital or survived reinfarction by four weeks

The patients were contacted during their stay in hospital by one of the physicians from the Postmyocardial Infarction Clinic At this first contact the patient was interviewed with respect to previous history of disease and symptoms These data were entered on computer records on which data from the present period of care were also registered At control examinations the patients were interviewed according to a previously designed form concerning the occurrence of symptoms since the previous control examination In addition ECG recordings were made

The χ^2 test was used to test differences between relative numbers Differences were considered statistically significant when $p<0.05$

RESULTS

Mortality

Table I shows the mortality by age and sex during two years follow up During the first year 12% of the men and 6% of the women had died after discharge The corresponding figures for two years follow up were 17% for men and 12% for women

Patients who suffered reinfarction had a higher mortality Of these 25% of the men and 13% of the women died during the first year The 2 year mortalities were 37% and 25% respectively The difference between men with primary infarctions and reinfarctions at entry to the study was statistically significant ($p<0.001$)

The percentage surviving at different points of time during the follow-up is shown in Fig 2 In the

Table I *Cumulative number of deaths during two years after myocardial infarction among patients with and without previous infarction*

	Men			Women			Total		
Age (y)	1 y	2 y	*N*	1 y	2 y	*N*	1 y	2 y	*N*
Total material (N=440)									
-39	0	0	9	0	0	1	0	0	10
40-44	5	6	27	1	1	3	6	7	30
45-49	10	13	71	0	2	9	10	15	80
50-54	9	14	112	0	0	13	9	14	125
55-59	5	6	60	1	1	19	6	7	79
60-64	8	16	59	1	2	18	9	18	77
65-67	5	5	21	2	4	18	7	9	39
Total	42 (12%)	60 (17%)	359	5 (6%)	10 (12%)	81	47 (11%)	70 (16%)	440
First infarction (N=364)									
-39	0	0	8	0	0	1	0	0	9
40-44	3	3	22	1	1	3	4	4	25
45-49	6	8	63	0	2	8	6	10	71
50-54	7	11	97	0	0	10	7	11	107
55-59	3	4	48	0	0	16	3	4	64
60-64	7	11	45	1	2	14	8	13	59
65-67	1	1	16	1	1	13	2	2	29
Total	27 (9%)	38 (13%)	299	3 (5%)	6 (9%)	65	30 (8%)	44 (12%)	364
Reinfarction (N=76)									
-39	0	0	1	0	0	0	0	0	1
40-44	2	3	5	0	0	0	2	3	5
45-49	4	5	8	0	0	1	4	5	9
50-54	2	3	15	0	0	3	2	3	18
55-59	2	2	12	1	1	3	3	3	15
60-64	1	5	14	0	0	4	1	5	18
65-67	4	4	5	1	3	5	5	7	10
Total	15 (25%)	22 (37%)	60	2 (13%)	4 (25%)	16	17 (22%)	26 (34%)	76

total material of men 17 had died after three months corresponding to 28% of the 2 year mortality After one year the figure corresponded to 70% of the 2 year mortality In the total material of women no deaths occurred during the first three months After one year half the deaths in women

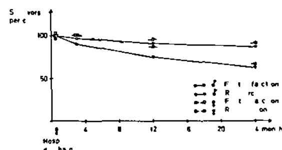


Fig 2 Percentage of survivors during two years after myocardial infarction among patients without and with previous infarction

had occurred (5/10) In men with primary infarctions 29% of the total 2 year mortality had occurred after three months One year after the infarction 71% of the total 2 year mortality in men with primary infarctions had occurred while for the women the corresponding figure was 50% (3/6) Six of the men with reinfarctions died during the first three months corresponding to 27% of the 2 year mortality After one year the proportions for men and women with reinfarction were 68% and 50% respectively (2/4)

The patients were also divided into one group below 55 years and one above The 1 year mortality was found to be 8% and 10% respectively in the men with primary infarctions their 2 year mortalities were 12% and 15% A similar situation was found for men with reinfarctions the mortality for those below 55 years being 38% (11/29) and for those above this age 35% (11/31) during two years follow up Thus this study did

Table II *Time between onset of symptoms and death together with place of death for deceased patients during two years after myocardial infarction*

	Men						Women		
	Total		First infarction		Re infarction		Total (n)	First infarction (n)	Re infarction (n)
	n	%	n	%	n	%			
Time between onset of symptoms and death (h)									
<1	28	47	16	42	12	55	4	2	2
<24	38	63	24	63	14	64	6	4	2
≥24	22	37	14	37	8	36	4	2	2
Total	60	100	38	100	22	100	10	6	4
Place of death									
Hospital	28	47	16	42	12	55	6	4	2
Outside hospital	32	53	22	58	10	45	4	2	2
Total	60	100	38	100	22	100	10	6	4

nite age-dependence with respect to mortality for all men men with primary infarctions or men with reinfarctions Owing to the low mortality no corresponding analysis could be made of the deaths in women

Interval between onset of symptoms and death and place of death

Table II shows the time elapsing between the onset of symptoms if any and death and the place of death for men and women Information concerning served or reported symptoms was available for deceased patients

Of the 60 deaths in the total material of men 28 (47%) occurred within one hour and 38 (63%) within 24 hours The remaining male patients died in hospital or after 1–22 days Of the 38 men with primary infarctions 16 (42%) died less than one hour after the onset of symptoms and 24 (63%) within 24 hours The remaining men with primary infarctions died in hospital or after 1–22 days Of the 22 deceased male reinfarction patients 12 (55%) died within one hour of the onset of symptoms and 14 (64%) within 24 hours Eight patients died after 2–19 days medical care In all 10 of the 22 deceased reinfarction patients died outside hospital

Of the total of 38 men who died within 24 hours of the onset of symptoms 32 (84%) died outside hospital The proportion of 24 hour deaths out

Table III *Causes of death during two years follow up after myocardial infarction for patients with and without previous infarction*

	Men						Women		
	Total		First infarction		Re infarction		Total (n)	First infarction (n)	Re infarction (n)
	n	%	n	%	n	%			
Fresh infarct	31	52	17	45	14	64	7	4	3
Other cardiovascular disease	4	7	3	8	1		1	1	0
No detectable cause	13	22	10	26	3	31	0	0	0
Progressive decompensation	5	8	2	5	3		1	0	1
Cardiac surgery	2	3	1	3	1		0	0	0
Suicide	3	5	3	8	0		0	0	0
Other	2	3	2	5	0		1	1	0
Total	60	100	38	100	22		10	6	4

Table IV *Post mortem findings during two years of follow up after myocardial infarction among patients with and without previous infarction*

	Men						Women		
	Total		First infarction		Re infarction		Total	First infarction	Re infarction
	n	%	*n*	%	*n*	%	(*n*)	(*n*)	(*n*)
Total	60	100	38	100	22	100	10	6	4
Autopsied	55	92	37	97	18	82	9	6	3
Heart weight g ($x \pm s_x$)	550±90		530±90		580±100		490±90	450±85	570±100
Degree of atherosclerosis (%)									
None	2	19	2	29	0		0	0	0
1–24	8		8		0		0	0	0
25–74	11	21	8	23	3		4	2	2
75–100	32	60	17	49	15	83	4	3	1
Total	53	100	35	101	18	100	8	5	3
Lumen obstruction									
None	5	60	4	69	1		1	1	0
Stenosis	27		20		7		3	1	2
Occlusion no fresh thrombosis	11	40	6	31	5		2	1	1
Fresh thrombosis	10		5		5		2	2	0
Total	53	100	35	100	18		8	5	3

side hospital was 92% for males with primary infarctions and 71% for males with reinfarctions The number of deaths in women was small as shown in Table II

Causes of death

The causes of death are listed in Table III In the total material of men cardiovascular diseases were *the cause of death in 89% Acute infarction was the* cause of death in 52% Seven patients (11%) died from non cardiovascular causes Two died in connection with open heart surgery the deaths being attributed to immediate complications of the operation it was considered that the patients would otherwise have survived longer possibly until the end of the study These deaths were therefore excluded from the cardiovascular group No other patients underwent cardiac surgery Three patients committed suicide one died of bacterial sepsis and one of advanced cancer of the stomach

In male patients with primary infarctions 84% of the deaths were due to cardiovascular diseases acute infarction being the cause of death in 45% For male reinfarction patients the corresponding figures are 95% and 64% respectively

In the total material of women nine of the ten deaths were caused by the cardiovascular diseases seven being the result of myocardial infarction One woman died of cancer of the bowel

Autopsy findings

Autopsy data are presented in Table IV In the total material of men 92% were autopsied The mean heart weight ±S D was 550±90 g Autopsy revealed *total occlusion of one of the three main* branches of the coronary artery in 40% being the result of fresh thrombosis in half of the patients In male patients with primary infarctions who later died arteriosclerotic engagement of more than 75% of the surface of the coronary vessels was found in 49% of the deceased Total occlusion was present in 31% being fresh in half of these No stenosis was found in 4 (14%) In men with reinfarctions who later died arteriosclerotic engagement of more than 75% of the surface of the coronary vessels was present in 83% Total occlusion was found in 56% of the patients and was due to fresh thrombosis in half of these One patient had no stenosis

The proportion of deceased men in whom autopsy revealed no cardiac or extracardiac cause of death and in whom death was therefore interpreted as being caused by arrhythmia was higher among

Table V Causes of death and autopsy findings among all male patients dying during two years after myocardial infarction according to time between onset of symptoms and death

	Time between onset of symptoms and death (h)			
	<24		≥24	
	n	%	*n*	%
Total	35		18	
Autopsied	33	94	17	94
Cause of death				
Fresh infarction	23	66	8	40
Other cardiovascular disease	1		3	20
No detectable cause	11	31	2	10
Progressive decompensation	0		5	30
Degree of atherosclerosis (%)				
<25	7	20	2	10
≥25	26	80	14	90
Lumen obstruction	9/32	30	7/17	40
Fresh thrombosis	6/32	20	3/17	25

the patients with primary infarctions (26%) than in male patients with reinfarctions (14%) (Table III)

In the total material of women the mean heart weight was 490±90 g Half of the patients (4/8) had rteriosclerotic engagement of more than 75% of surface of the coronary vessels None was free arteriosclerosis Four had occlusions and sh thrombosis was found in two of these One woman had no stenosis Women with primary infarctions had a mean heart weight of 450±85 g compared with 570±100 g in women with reinfarctions No meaningful analysis could be made of the extent of arteriosclerosis stenosis and occlusions in women with primary infarctions and reinfarctions owing to the small numbers involved No woman died without demonstrable cause

The incidence of infarction scars and myocardial fibrosis was slightly higher in patients with reinfarctions in both men and women but no significant differences were found

Causes of death and autopsy findings in men who died from cardiovascular diseases within 24 hours of the onset of symptoms

In this section all men who died from cardiovascular diseases during two years follow up within 24 hours of the onset of symptoms if any ($n=35$) are compared with those who died after a longer interval ($n=18$) (Table V) As an analysis of the males with primary infarctions gave similar results the presentation comprises all men Only three of the patients included in this comparison were not autopsied

Among the patients who died within 24 hours no direct cause of death was found at autopsy in one third compared to one tenth of those who died after more than 24 hours Morphological evidence of fresh infarction was more common among those who died within 24 hours—70% (23/35) compared to 40% (8/18) for those who died later In the group of patients who died after more than 24 hours death due to other cardiovascular diseases including progressive decompensation was considerably more common

No distinct differences between the groups were found with respect to degree of arteriosclerosis lumen occlusion infarction scars or diffuse myocardial fibrosis

Non fatal reinfarctions

In men with primary infarctions ($n=299$) 8% suffered reinfarction during the first year and 12% during the first two years Of the male patients who suffered reinfarction at entry to the study ($n=60$) 22% had non fatal reinfarctions during the first year and 27% during the first two years For the women with primary infarctions ($n=65$) the corresponding figures were 6% and 14% respectively Four of the 16 women who entered the study with reinfarctions suffered non fatal reinfarction during the first year of follow up and none during the second year (Table VI)

The percentages of the men who had not suffered non fatal reinfarction at various points of time during the follow-up are shown in Fig 3 The incidence of non fatal reinfarction was significantly higher during the first year compared to the second year of follow up ($p<0.025$) In the total material of men 11 (22%) of the reinfarctions during two years follow up occurred within three months and 39 (76%) within 12 months The corresponding figures for men with primary infarctions were 9 (26%) and 26 (74%) respectively In men with reinfarctions 13% (2/16) of the non fatal reinfarctions during two years occurred within three months and 81% (13/16) within one year

No age-dependence could be demonstrated for the men The incidence of non fatal reinfarctions

Table VI *Cumulative number of non fatal reinfarctions during two years after myocardial infarction among patients with and without previous infarction*

	Men			Women		
Age (y)	1 y	2 y	*N*	1 y	2 y	*N*
Total material (*N*=440)						
–39	2	2	9	0	0	1
40–44	1	3	27	0	0	3
45–49	8	9	71	1	3	9
50–54	12	17	112	0	0	13
55–59	7	11	60	3	3	19
60–64	6	7	59	0	0	18
65–67	2	2	21	4	7	18
Total	*32 (11%)*	*51 (14%)*	*359*	*8 (10%)*	*13 (16%)*	*81*
First infarction (*N*=364)						
–39	1	1	8	0	0	1
40–44	0	2	22	0	0	3
45–49	4	5	63	0	2	8
50–54	10	14	97	0	0	10
55–59	6	8	48	2	2	16
60–64	3	4	45	0	0	14
65–67	1	1	16	2	5	13
Total	25 (8%)	35 (12%)	299	4 (6%)	9 (14%)	65
Reinfarction (*N*=76)						
–39	1	1	1	0	0	0
40–44	1	1	5	0	0	0
45–49	4	4	8	1	1	1
50–54	2	3	15	0	0	3
55–59	1	3	12	1	1	3
60–64	3	3	14	0	0	4
65–67	1	1	5	2	2	5
Total	13 (22%)	16 (27%)	60	4 (30%)	4 (30%)	16

was the same for those below and above 55 years of age respectively Nor was any age-dependence found for patients who had suffered reinfarction at entry to the study Among the women reinfarction was uncommon and no detailed analysis was possible

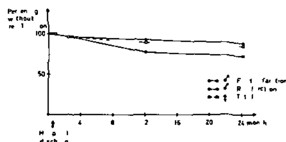


Fig 3 Percentage of patients without non-fatal reinfarctions during two years after myocardial infarction among patients with and without previous infarction

A second non fatal reinfarction during the follow up period occurred in only five individuals all of them men with primary infarction These five were all above 55 years of age and survived for the remainder of the period of follow up

In men with primary infarctions who later suffered non fatal reinfarction (n=35) 5 (14%) died during the remaining period of follow up on an average 3 8 months after the new reinfarction Of the patients with reinfarctions who later suffered non fatal reinfarctions (n=16) 5 (30%) died before the end of the period of follow up on an average 6 6 months after the new reinfarction

DISCUSSION

A number of reports concerning the survival of infarction patients after discharge from have been published (5 6 10 23

59) Many studies have demonstrated 1 year mortalities of the order of 10–20% (25 26 40 44 53 54) During the second year of follow up the mortality is of the order of 3–15% in several of these series All the studies referred to have certain limitations which complicate comparisons between materials as discussed by Skall Jensen (54) and Seigel and Loncin (52) Lew (34) found that the differences between several of these studies were due to discrepancies with respect to age sex previous infarction and socio-economic factors and he therefore pointed out the need for studies in representative materials

The present study covers all deaths and non fatal reinfarctions during two years follow up in patients in the population of Goteborg who survived their stay in hospital after infarction with the limitations stated in the patient description The population of Göteborg is geographically very stable in these age groups The investigation was performed prospectively and the patients were cared for by a small group of physicians who had trained together—the physicians at the Postmyocardial Infarction Clinic (19 55 63) Thanks partly to this no patient dropped out of the study during the follow up period Various clinical variables for the patients participating in the study have been presented previously (56)

During 1968–70 half of the men participated in a dy of the effects of supervised physical training and during 1970 63 men and women were aced on alprenolol in a study of the effects of chronic β blockade (65) Both these studies yielded some reduction of the mortality For patients participating in the present study and for the follow up period of two years concerned here this had no significant influence on the incidences reported as shown by a comparison of reinfarction and mortality between the present study and the control groups in the studies referred to above As a result of the participation of certain patients in these studies the patients with a first infarction who participated in the present investigation had a maximum reduction of mortality of about three cases in the total material or 0 7% This influence on the incidences reported has been accepted since the total effect on the material was not great and the possible divergence could be calculated

Adequate classification of causes of death especially with regard to sudden death necessitates a high incidence of autopsies as pointed out by for example Wikland (62) In the present study the total incidence of autopsies was 92% Even when the incidence of autopsies is high it is important that attention is paid to the direct classification of causes of death It cannot be taken for granted that deaths for which no cardiac or other cause of death can be demonstrated can be assigned to acute infarction or ischaemic heart disease even when coronary heart disease is found at autopsy (51) In the present study deaths for which no direct cause could be found have been assigned to the group cardiovascular diseases on the assumption that they were due to arrhythmias since with the routine methods at present available it is not possible to pursue the diagnosis of morphological infarction further In this material all autopsied cases of infarction were classified as certain (Fig 1 A I) Thus a minimum incidence of fresh infarction was recorded

Three large studies have reported 1 and 2 year mortalities which accord well with the present findings (3 44 53) Norris et al (40) in a material of 530 patients aged up to 90 years reported a mortality of 16% during the first year and 25% during the first two years which is higher than in the present study All the studies referred to have demonstrated a higher mortality during the first year after discharge from hospital compared to later points of time during the follow up When the mortality during the first year in the present material was studied separately it was found to be higher during the first few months after discharge as has been reported in several of these studies

The age-adjusted mortality in ischaemic heart disease is considerably lower in women than in men (58) Several studies have not however demonstrated any sex differences in long term mortality after infarction (23 24 41 61) Other studies even suggest a poorer relative long term survival for women than for men (10 27 53 54) No certain differences in mortality between men and women were found in the present study

It has been shown in several reviews that most studies report an increasing long term mortality with age (33 34 36) No age trend was found in the present material either when age was analysed separately or when it was included in a multivariate analysis (56) Peel (42) found that the mortality did not increase until above the age of 60 The total mortality in ischaemic heart disease (58) and the mortality prior to admission to hospital (32 64) also

increase with age In the present study all patients were routinely cared for at the Postmyocardial Infarction Clinic Observations have been presented which suggest that this after-care can reduce the morbidity and mortality (57) This together with the fact that the upper age limit was set at 67 years and that the period of follow up was limited to two years may explain the lack of age-dependence with respect to long term mortality Similar findings have been presented by other authors (38)

The significance of having suffered previous infarction has varied in different studies It is reasonable to suppose that the prognosis will be worse in patients with reinfarction since the total cardiac damage increases It has been demonstrated that there is a correlation between cardiac damage and prognosis (16 24 40 56) The finding that the prognosis is poorer in patients with reinfarction is supported by several studies (11 26 33 35 40 60) Other authors have however interpreted their findings as showing that previous infarction does not influence the long term prognosis (24 41) In these studies the mean age was higher than in the present investigation

In the present study the proportion of patients who died shortly after the onset of symptoms is high Other studies have also shown a high incidence of deaths during the first 24 hours after the onset of symptoms (2 13 66) Direct comparison is difficult since definitions vary and sudden death is often used to include deaths which are not witnessed

More than half of the patients in the present study died outside hospital The proportion was lower in reinfarction patients which may reflect their greater awareness of the need for medical care when symptoms occur Death in ischaemic heart disease often occurred outside hospital as has been found in several other studies (19 21 32 62)

It was found that 92% of the deaths in the total male material were attributable to cardiovascular diseases and the remaining five to other causes Several other studies have shown that 80–90% of deaths in infarction patients during follow-up are the result of cardiovascular diseases (1 10 27 54 60) Of the total number of deaths in men aged 35–65 years in Sweden almost half are due to cardiovascular diseases (58)

The occurrence of coronary sclerosis and coronary atheromatosis in patients with myocardial infarction has been well studied (9 47 50 68) Although many expect to find a coronary thrombosis in fatal myocardial infarctions the incidence reported varies within such wide limits as 7–91% (46) The differences are due to differences in patients methods and definitions The role of thrombosis in acute myocardial infarction is in fact far from clear (20 22 46)

In a methodologically sophisticated multicentre study under the auspices of the WHO in which the pathologists performing the autopsies trained together a subgroup of patients was found in which the large extramyocardial coronary vessels were free from thrombosis or other occlusions This subgroup revealed an increased incidence of sudden deaths and a reduced incidence of the highest degree of coronary atheromatosis (28) In contrast to this another study revealed a higher incidence of coronary vascular changes in persons who died immediately compared to others who died within 24 hours (22) In the present study arteriosclerosis and narrowing of the lumen was equally common in all patients irrespective of the interval between the onset of symptoms and death It should be noted that this material comprised patients who had previously suffered at least one clinical infarction while other studies included some individuals with sudden death as the first manifestation of ischaemic heart disease Note also that different pathologists who had not trained together performed the autopsies in the present study Patients with several previous infarctions had more coronary vascular changes and higher heart weights It may therefore be expected that the difference with respect to arteriosclerosis and thrombosis between those who died rapidly and other deaths will decrease with an increasing number of previous infarctions

The 2 year mortality in a representative material of patients who survived their stay in hospital is thus of the same order as the mortality during the acute phase The risk of sudden death is great These findings have led to efforts to identify individuals with a high risk of dying after myocardial infarction (24 26)

The combination of infarction registration which has been validated separately (19) and a Post myocardial Infarction Clinic with negligible drop outs (15 18) makes it possible to analyse the occurrence of non fatal reinfarctions as a step towards the description of the natural history of ischaemic heart disease

Different factors have been shown to be as

sociated with death and non fatal reinfarction during the follow up of infarction patients (56) It is therefore important to study fatal and non fatal reinfarctions separately

In only a few studies has a distinction been made between these two types of reinfarction (10 14 25) In these the incidences of non fatal reinfarction during follow up were lower than in the present study but there are considerable differences between the studies with respect to patients and methods

No age trend for the occurrence of non fatal reinfarction was found in the present study although there was a tendency for the incidence of recurrences to be higher in reinfarction patients up to the age of 55 years Similar findings have been reported by other authors (53 54) Weinblatt et al (59) found that the risk of a first reinfarction increased with age Below 55 years the incidence was 22% and above this age it was 28% among patients who survived the acute phase and were followed up for on an average 4 5 years In the study by Weinblatt et al relative risk compared to the normal population was greater for men below than above 55 years of age The same was found in the present investigation since the incidence of infarction increases with increasing age in the normal population

During the first year of follow up the number of non fatal reinfarctions was significantly higher than during the second year for men in both the total material and the material of primary infarctions and reinfarctions This is in accordance with findings reported in the literature (8 53 59)

The risk of a new non fatal reinfarction seems to increase with the number of previous clinical infarctions The feared negative influence of a non fatal reinfarction on the long term prognosis is confirmed by the observation that the mortality for the patients who entered the study with primary infarctions and who later suffered non fatal reinfarctions was high in relation to the short remaining period of follow up This finding is also valid for patients with reinfarction at entry to the study This observation is supported by previous findings (35)

The mortality and the occurrence of non fatal reinfarctions was not substantially lower for women in the present study than for men On the other hand it is well known that the incidence of infarction in previously healthy individuals in these groups is considerably lower among women than among men (4, 12 19, 48) The similar incidence of non fatal reinfarctions and cardiovascular deaths in the two sexes after myocardial infarction may indicate that once these individuals have suffered infarction they are exposed to the same risk and, perhaps the same pathogenetic mechanisms tending to lead to a second infarction

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Table I *Clinical data on the 25 patients examined*

Pat no	Age (y)	Sex	Duration of hyper tension (mo)	BP (mmHg)	Changes in optic fundi	Increased cardio/ thoracic ratio	LV hypertrophy or strain pattern	Cr clearance (ml/min)	Preceding treatment
1	35	♀	30	170/120	1	0	0	110	+
2	24	♂	7	180/120	1	0	+	99	0
3	24	♂	2	160/110	0	0	0	135	0
4	22	♀	4	180/120	0	0	+	142	0
5	21	♂	6	150/110	0	0	0	124	0
6	30	♀	3	160/110	2	0	0	145	0
7	25	♂	9	150/110	0	0	0	100	0
8	29	♂	0	170/115	1	+	+	115	0
9	21	♀	20	160/115	1	+	+	105	+
10	28	♂	1	160/110	1	0	+	105	0
11	48	♀	60	200/130	2	0	0	118	+
12	43	♀	132	210/125	2	+	0	75	+
13	38	♂	14	160/125	1	0	0	95	+
14	58	♀	2	170/110	1	+	0	95	0
15	48	♀	19	200/110	2	0	+	88	+
16	38	♀	60	180/120	1	+	0	105	+
17	51	♂	7	190/120	3	0	+	65	+
18	52	♂	3	210/130	2	+	+	76	0
19	53	♂	48	180/115	2	+	+	104	+
20	59	♂	108	210/130	2	0	+	92	+
21	57	♂	2	190/125	2	+	+	100	0
22	52	♂	3	180/115	2	0	+	130	0
23	49	♂	24	180/115	2	0	+	81	+
24	65	♀	108	240/150	2	+	+	105	+
25	53	♂	4	220/125	2	+	+	80	0

hypertonicus I and one with fundus hypertonicus II In group 2 there were 3 patients with fundus hypertonicus I 11 with fundus hypertonicus II and one with fundus hypertonicus III Five patients in group 1 dispalyed left entricular (LV) hypertrophy electrocardiographically d two of these had an increased cardio/thoracic ratio In group 2 there were 10 patients with LV hypertrophy and/or strain pattern and 8 patients with an increased cardio/thoracic ratio Kidney function was normal in both groups and no patients had proteinuria Creatinine clear ance was on an average higher in group 1 (118 ml/min) than in group 2 (94 ml/min) Hypertension had been known for an average of 8 months in group 1 compared to 40 months in group 2 Among the patients in group 1 only 2 had previously received antihypertensive treatment whereas 10 patients in group 2 had been treated earlier

All patients were screened to exclude the possibility of secondary hypertension None exhibited clinical radiological or biochemical signs of kidney disease disorders in renal arteries adrenal disorders or coarctatio aortae

All patients were informed of the nature of the tests prior to the start of the study and all agreed to participate

Procedure

All patients were out-patients and were systematically screened once a week for 2 months The first month was a control period during which all medical treatment was discontinued During the second month the patients were treated with alprenolol (Aptin® capsules) The average dosage in group 1 was 900 mg/day and in group 2 1093 mg/day Clearance measurements were performed at the

Table II *Comparison of age blood pressure changes in optic fundi (FH) creatinine clearance and duration of hypertension between groups 1 and 2 (mean values)*

	Age (y)	Duration of hyper tension (mo)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Changes in optic fundi	Cr clearance (ml/min)
Group 1	25 9	8	164	114	0 5	118
Group 2	50 9	40	195	123	1 9	94
p	<0 01	n s	<0 01	<0 02	<0 01	n s

n s =not significant

Table III *Blood pressure (mmHg) and heart rate (beats/min) before (B) and after (A) treatment with alprenolol for the patients in groups 1 and 2*

Pat no	Systolic BP		Diastolic BP		Mean BP		Heart rate	
	B	A	B	A	B	A	B	A
Group 1								
1	170	160	120	110	137	127	83	67
2	180	135	120	95	140	108	71	63
3	160	130	110	90	127	103	79	61
4	180	150	120	110	140	123	83	63
5	150	135	110	95	123	108	59	47
6	160	135	110	95	127	108	77	64
7	150	125	110	85	123	98	71	63
8	170	160	115	110	133	127	55	55
9	160	140	115	100	130	113	59	65
10	160	140	110	95	127	110	63	63
Mean	164	141	114	99	131	113	70	61
S D	10 7	12 0	4 6	8 8	7 3	10 0	10	5 9
S E M	3 4	3 8	1 5	2 8	2 3	3 2	3 3	1 9
p		<0 01		<0 01		<0 01		<0 01
Group 2								
11	200	190	130	115	153	140	69	63
12	210	190	125	115	153	140	63	67
13	160	130	125	100	137	110	63	55
14	170	170	110	110	130	130	63	55
15	200	190	110	110	140	137	63	55
16	180	170	120	115	140	133	79	79
17	190	170	120	110	143	130	79	69
18	210	190	130	120	157	143	71	65
19	180	180	115	110	137	133	61	55
20	210	180	130	120	157	140	67	55
21	190	180	125	130	137	147	81	59
22	180	170	115	115	137	133	75	71
23	180	140	115	95	137	110	63	59
24	240	200	150	120	180	147	67	63
25	220	200	125	125	158	150	71	67
Mean	195	177	123	114	146	135	69	62
S D	21 0	19 9	10 1	8 9	13 0	12 0	6 8	7 3
S E M	5 4	5 1	2 6	2 3	3 4	3 1	1 7	1 9
p		<0 01		<0 01		<0 01		<0 01

end of the control period and after one month s treatment with alprenolol

Measurements

BP was measured after at least 1 hour s rest in the supine position immediately prior to clearance measurements BP measurements were carried out using the same sphygmomanometer and were repeated several times on each occasion The lowest reading was used

Clearance measurements were performed using the constant infusion technique (8 20) The reference substances employed were [131]I hippuran and [125]iothalamate (obtained from Amersham Radiochemical Pharmaceuticals) The labelled substances were stored in the dark at 4°C for a maximum of 2 weeks The amount of free iodine was less than 2% A priming dosage was given so that the activity in plasma was 300–600 cpm/ml for [131]I hippuran and 800–1600 cpm/ml for [125]iothalamate Plasma activity was kept stable by constant infusion with the aid of a Holter infusion pump The measurements of radioactivity were performed in a dual-channel analyser to a statistical inaccuracy of less than 1% Each patient was examined for 3 or 4 clearance periods of approximately 30 min Blood specimens were taken 5 min before the middle of each clearance period Bladder catheterization was not performed The patients urinated in either standing or sitting positions In the course of 1 hour immediately prior to examination 1000 ml water was given to each patient and during the actual clearance periods a further 500 ml/h Earlier investigations (13 19) have shown that [131]I hippuran and [125]iothalamate are reliable reference substances for the measurement of RPF and GFR

Calculations

All clearances of [131]I hippuran and [125]iothalamate have been corrected to a standard surface of 1 73 m² Mean BP

Table IV *Comparison between groups 1 and 2 before and after treatment with alprenolol (mean values)*

C_{131I-h}=clearance of [131]I hippuran $C_{125I_{io}}$=clearance of [125]iothalamate $C_{125I_{io}}/C_{131I-h}$=filtration fraction RVR=renal vascular resistance

	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean BP (mmHg)	Heart rate (beats/min)	C_{131I-h}	$C_{125I_{io}}$	$C_{125I_{io}}/C_{131I-h}$	RVR
Before treatment								
Group 1	*164*	*114*	*131*	*71*	*443*	*113*	*0 261*	*0 187*
Group 2	195	123	146	70	362	98	0 276	0 270
p	<0 01	<0 02	<0 01	n s	n s	n s	n s	<0 02
After treatment								
Group 1	141	99	113	62	468	122	0 266	0 150
Group 2	177	114	135	63	319	89	0 285	0 276
p	<0 01	<0 01	<0 01	n s	n s	n s	n s	<0 01

n s =not significant

has been calculated by adding 1/3 of the pulse pressure to diastolic BP The renal blood flow was calculated using RPF and venous hematocrit The RVR was determined by dividing mean BP by RPF

Mann Whitney s rank sum test was used for statistical processing when comparing groups 1 and 2 Changes in BP pulse frequency RPF GFR filtration fraction and RVR were compared using Wilcoxon s signed rank test Spearman s test was used to calculate correlations

RESULTS

lood pressure and heart rate

he results for both groups are given in Table III *In group 1 alprenolol treatment resulted in a* statistically significant lowering of the BP Systolic BP was diminished from 164 to 141 mmHg ($p<$ 0 01) diastolic BP from 114 to 99 mmHg ($p<$0 01) *and mean* BP from 131 to 113 mmHg ($p<$0 01) Three patients (nos 1 4 8) had a diastolic BP of 110 mmHg after treatment The remainder had diastolic BP of 100 mmHg or less Heart rate fell from 70 to 61 beats/min ($p<$0 01)

In group 2 alprenolol induced a statistically significant lowering of BP Systolic BP was reduced from 195 to 177 mmHg ($p<$0 01) diastolic BP from 123 to 114 mmHg ($p<$0 01) and mean BP from 146 to 135 mmHg ($p<$0 01) Only two patients (nos 13 23) had diastolic values of 100 mmHg or less after treatment For the other patients in group 2 the diastolic BP in spite of a significant reduction was still 110 mmHg or higher after treatment The heart rate was lowered from 69 to 62 beats/min ($p<$0 01)

The average BP level was significantly higher in group 2 than in group 1 before treatment This difference was still present after alprenolol treatment (Table IV) There was no difference in heart rate either before or after treatment between groups 1 and 2

Renal plasma flow glomerular filtration rate filtration fraction and renal vascular resistance

The results for both groups are given in Table V In group 1 RPF changed during alprenolol treatment from 443 to 468 ml/min GFR altered from 113 to 122 ml/min and the filtration fraction was 0 261 before and 0 266 after treatment These minimal *differences were all insignificant* ($p>$0 05) *However* RVR was reduced significantly after alprenolol treatment from 0 187 to 0 150 ($p<$0 05)

In group 2 RPF changed from 362 to 319 ml/min GFR changed from 98 to 89 ml/min The filtration fraction was 0 270 before and 0 285 after treatment These differences were all insignificant There was no change in RVR 0 270 before and 0 276 after treatment ($p>$0 05)

In Table IV RPF GFR filtration fraction and RVR in groups 1 and 2 are compared before and after alprenolol treatment No differences were found in RPF GFR or filtration fraction RVR however was significantly lower in group 1 than in group 2 both before ($p<$0 02) and after treatment ($p<$0 01)

Correlations

There is no correlation between the change in mean BP and the changes in RPF and GFR There is a

Table V *Clearances of ¹³¹I hippuran and ¹²⁵I iothalamate filtration fraction and renal vascular resistance before (B) and after (A) treatment with alprenolol in the patients in groups 1 and 2*

Abbreviations as in Table IV

Pat no	C_{131I-h} B	C_{131I-h} A	C_{125Io} B	C_{125Io} A	C_{125Io}/C_{131I-h} B	C_{125Io}/C_{131I-h} A	RVR B	RVR A
Group 1								
1	379	487	110	156	0 290	0 320	0 217	0 157
2	267	315	82	82	0 207	0 260	0 315	0 206
3	422	582	98	103	0 232	0 177	0 181	0 106
4	425	608	126	159	0 297	0 262	0 201	0 123
5	537	539	118	115	0 220	0 213	0 133	0 116
6	383	320	111	103	0 290	0 322	0 206	0 209
7	454	500	123	117	0 271	0 234	0 165	0 120
8	503	479	127	141	0 253	0 294	0 151	0 151
9	599	413	117	127	0 195	0 308	0 128	0 161
10	457	438	117	116	0 256	0 265	0 172	0 156
Mean	443	468	113	122	0 261	0 266	0 187	0 150
S D	*92 2*	*99 1*	*13 8*	*24 4*	*0 037*	*0 048*	*0 054*	*0 036*
S E M	29 2	31 3	4 4	7 7	0 012	0 015	0 017	0 011
p		>0 05		>0 05		>0 05		<0 05
Group 2								
11	334	359	107	121	0 320	0 337	0 289	0 246
12	305	330	81	98	0 266	0 297	0 301	0 255
13	293	377	94	103	0 321	0 273	0 281	0 175
14	369	434	69	90	0 187	0 207	0 226	0 192
15	557	233	145	68	0 260	0 292	0 158	0 370
16	629	472	133	122	0 211	0 259	0 138	0 175
17	259	250	68	57	0 262	0 228	0 364	0 343
18	358	259	91	73	0 254	0 281	0 272	0 342
19	334	246	88	81	0 264	0 329	0 270	0 357
20	242	210	73	66	0 302	0 314	0 396	0 400
21	498	339	119	86	0 239	0 254	0 138	0 252
22	393	364	118	105	0 300	0 288	0 216	0 227
23	265	232	84	85	0 317	0 366	0 305	0 279
24	276	305	98	93	0 355	0 305	0 424	0 313
25	324	379	96	94	0 296	0 248	0 266	0 217
Mean	362	319	98	89	0 276	0 285	0 270	0 276
S D	113	79	23	19	0 045	0 042	0 086	0 074
S E M	29 4	20 5	5 9	4 9	0 012	0 011	0 022	0 019
p		>0 05		>0 05		>0 05		>0 05

significant correlation between mean BP after alprenolol treatment and age (ϱ=0 7779 p<0 001) There is no correlation between BP after treatment and the known duration of increased BP

DISCUSSION

The results show in accordance with other investigations (1 2 3 4 7 22) that alprenolol induces a significant lowering of BP in patients with arterial hypertension

The mechanism by which β adrenergic blocking agents act in reducing BP is still unsolved Several investigators (5 6) have shown that both alprenolol and propranolol cause a significant reduction of plasma renin activity in patients with essential hypertension and it has been suggested that lowering of plasma renin activity by β adrenergic agents is the reason for the antihypertensive effect Other researchers (16) believe that the lowering of BP is a result of the decrease in cardiac output which continuously accompanies β adrenergic blockade However an effective lowering of the BP occurs only in patients in whom the peripheral resistance is not increased (21) The reason for the reduction of BP is believed to be a resetting of the baroreceptors whereby BP is adjusted at a lower level (15)

In the present study there is a markedly better BP

reduction in group 1 than in group 2 even though there was no significant difference in heart rate before and after treatment between the two groups The patients in group 1 differ from those in group 2 in that they are younger the duration of their hypertension is shorter they have a lower BP level and exhibit less marked hypertensive organic lesions The cardiac output is usually increased in the early stages of hypertension (12) In hypertension of longer duration however the peripheral resistance is increased and the cardiac output is low or normal The difference between BP reduction in the two groups could be due to the fact that a resetting of the baroreceptors is more difficult when cardiac output is low beforehand and that a secondary change in BP to a lower level is possibly more difficult due to a greater degree of structural vascular alterations It is in accordance with this theory that mean BP after treatment is positively significant correlated to the patient s age On the other hand there is no correlation between duration of hypertension and mean BP after treatment This result is however disputable since there frequently exists a discrepancy between the known and the actual duration of the hypertensive disease

The effect of the various antihypertensive agents on GFR and RPF has been studied previously Judson et al (10) found an increase in RPF and GFR in a number of patients with essential hypertension after hydralazine treatment whereas decreased values were found during guanethidine treatment (17) The reason for this discrepancy is believed to be the difference in the hemodynamic effect of these two antihypertensive agents Hydralazine increases (10) while guanethidine reduces cardiac output (17) Since β adrenergic blocking agents continuously lower the cardiac output one would expect that RPF and GFR would also be reduced during β adrenergic blockade The results from this study however show that there is no significant difference in RPF and GFR before and after treatment

Very few reports exist on the effect of β adrenergic blocking agents on RPF and GFR Schirmeister et al (18) measured a 10–15% reduction in the clearance of para aminohippuric acid and inulin in 22 patients with arterial hypertension after infusion of propranolol During infusion no alterations were registered in BP Although this could point to a direct renal influence of propranolol no changes could be measured in GFR and RPF following injection of a β adrenergic stimulating agent These researchers therefore assumed that β adrenergic receptors do not affect the renal blood flow in patients with hypertension

In animal experiments Winer et al (23) measured GFR and RPF in normotensive dogs after i v infusion of propranolol However they found no change in RPF and GFR after injections of propranolol into the renal artery enabling them to measure RPF and GFR for each kidney individually It was therefore concluded that the effect of propranolol on kidney function was the result of its action on the circulation as a whole and in particular a consequence of reduced cardiac output The animal experiments and those of Schirmeister et al differ in several respects from the present study The measurements were performed immediately after infusion the agent was given i v and a different β adrenergic blocking agent was used

Krauss et al (11) measured renal blood flow with the aid of ^{125}I hippuran and renal vascular resistance in 9 patients with essential hypertension before and after a minimum of 10 days treatment with propranolol and they found a 14% decrease in renal blood flow and a 13% increase in RVR Only one of their patients was under 35 years of age and their material is therefore comparable to group 2 in the present study We also measured a decrease in RPF and an increase in RVR but these alterations were not significant

Ibsen and Sederberg Olsen (9) studied the effect of propranolol treatment on ^{51}Cr EDTA clearance in 20 patients with arterial hypertension Twelve patients displayed a 13% reduction of ^{51}Cr EDTA clearance after 2–3 and 4–5 months propranolol treatment In 8 patients a 15% increase in ^{51}Cr EDTA clearance was seen 2 months after discontinuation of propranolol treatment Only one patient was under 35 years and the material is comparable to group 2 in the present study In the present investigation a slight decrease in GFR was measured but this change was not significant

When RVR was calculated it was found to be significantly lower for group 1 than for group 2 both before and after alprenolol treatment RVR was markedly reduced after alprenolol treatment in group 1 whereas no alterations were found in group 2 following treatment Since alprenolol treatment did not cause alterations in RPF the difference in RVR must primarily be due to a reduction of mean BP

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Catecholamines in Plasma and Urine in Patients with Essential Hypertension Determined by Double-Isotope Derivative Techniques

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ABSTRACT Employing double-isotope derivative techniques, noradrenaline and adrenaline have been determined in plasma and in urine and dopamine in urine in 21 patients with essential hypertension as well as in 32 controls Plasma noradrenaline rose with age in both groups of subjects No differences were observed in plasma noradrenaline and plasma adrenaline in the resting supine position and in urinary excretion of noradrenaline and dopamine in hypertensive patients as compared to control subjects Urinary excretion of adrenaline was somewhat lower in the hypertensives than in the controls Treatment with alprenolol, a β adrenergic blocking agent, did not influence noradrenaline and adrenaline in plasma in the basal state or the urinary excretion of the three catecholamines The combined treatment with alprenolol and hydralazine was followed by a significant rise in plasma noradrenaline It is concluded that the adrenergic activity evaluated by circulating catecholamines is normal in most patients with essential hypertension

Most patients with hypertension are treated with drugs which inhibit the activity of the adrenergic system An increased adrenergic activity has often been implicated in the pathogenesis of essential hypertension although no well defined relationship has been established

Studies of urinary excretion of noradrenaline and adrenaline have not revealed any consistent abnormality in most patients with essential hypertension (2 8 11 14 17) while the excretion of dopamine has been reported to be normal or decreased (10 13 19)

Employing a double isotope derivative technique for the determination of noradrenaline and adrenaline several groups have recently reported elevated plasma catecholamine concentrations in patients with essential hypertension (9 12 15 16) In a previous study (3) total plasma catecholamine concentration was measured in a small number of patients with renal and essential hypertension but no abnormality was found Due to this discrepancy we have measured plasma noradrenaline and *plasma adrenaline as well as urinary excretion of* noradrenaline adrenaline and dopamine in a larger number of patients with essential hypertension

Plasma noradrenaline and adrenaline concentrations were also measured in some patients after a period of treatment with alprenolol as well as after combined treatment with alprenolol and hydralazine Urinary excretions of the three catecholamines were examined before and after treatment with alprenolol

SUBJECTS

A total of 21 hypertensive patients and 32 controls were examined All patients had essential hypertension Secondary hypertension was ruled out by clinical examination determinations of serum electrolytes creatinine clearance and urinary protein excretion An excretory pyelogram was also performed

At the initial examination all hypertensive patients were without treatment 12 had never received antihypertensive therapy 9 had earlier been treated with methyldopa or thiazides The antihypertensive therapy had been withdrawn at least 4 weeks before the examination

Creatinine clearance averaged 95 ml/min (range 45-145) in the hypertensive patients None of the patients had permanent proteinuria Three patients had no retinal changes 5 had fundus hypertonicus I 11 fundus hypertonicus II and 2 fundus hypertonicus III Eleven patients had signs of left ventricular hypertrophy or strain on the ECG None of the patients had cardiac insufficiency

Table I *Plasma noradrenaline and plasma adrenaline concentrations and blood pressures in patients with essential hypertension and in control subjects*

	Controls		Hypertensives	
	Males	Females	Males	Females
N	26	6	9	10
Plasma noradrenaline (ng/ml)				
Mean	0 258	0 238	0 256	0 229
Range	0 08–0 75	0 12–0 43	0 08–0 43	0 13–0 34
Plasma adrenaline (ng/ml)				
Mean	0 045	0 058	0 048	0 021
Range	0 00–0 16	0 02–0 19	0 01–0 15	0 01–0 04
Age (y)				
Mean	40 3	39 8	42 7	39 5
Range	20–65	23–65	24–59	22–57
Systolic BP (mmHg)				
Mean	127	123	188	189
Range	100–160	100–160	150–220	160–220
Diastolic BP (mmHg)				
Mean	84	78	121	117
Range	70–100	70–90	110–130	100–130

The following groups of patients were studied 1) Plasma noradrenaline and plasma adrenaline were measured in 19 patients 10 females and 9 males with untreated essential hypertension The mean age was 41 years (range 22–59) 2) Urinary excretions of noradrenaline adrenaline and dopamine were measured in 10 untreated patients 6 females and 4 males The mean age was 50 years (range 29–69) 3) Plasma noradrenaline and adrenaline were measured in 10 hypertensive patients after 4 weeks of [illegible] with alprenolol (1200 mg a day divided into 3 [illegible]) and again 4 weeks after the combined treatment [illegible] alprenolol and hydralazine (160 mg a day divided into [illegible]es) 4) Urinary excretion of the catecholamines was reexamined in 10 patients 4 weeks after treatment with alprenolol

The following groups of control subjects were examined 1) Plasma noradrenaline and plasma adrenaline were measured in 32 controls 6 females and 26 males The mean age was 40 years (range 20–65) 2) Urinary excretion of catecholamines was measured in 10 controls 4 males and 6 females The mean age was 47 years (range 27–66) None of the control subjects had symptoms of cardiovascular disease and none of them received drugs known to influence the adrenergic system

PROCEDURE

Blood samples were drawn in the morning after at least 30 min rest in the supine position The subjects had fasted for approximately 8 hours Smoking was prohibited but water intake was not limited

Blood 10–20 ml was collected from an antecubital vein via an indwelling catheter and when at least 15 min had elapsed between the insertion of the catheter and the first blood collection Blood was collected in ice-cooled tubes containing ascorbic acid and EDTA and treated as described previously (5) The BP was measured just before collection of the blood samples

The urine was collected in containers and immediately acidified The 24 hour urine volume was measured and a sample of approximately 5 ml was frozen until the analysis

METHODS

Noradrenaline and adrenaline were measured by a double isotope derivative technique (5) 4–10 ml plasma and 100–200 μl urine being used in the analysis

The dopamine concentration in urine was measured by a double isotope derivative technique described previously (6 7) Approximately 25–50 μl of the urine was used in the analysis

Regression analysis was performed according to Bailey (1) Student s *t* test the Mann Whitney *U* test and the Wilcoxon test were used for comparison of means (1 18)

RESULTS

Table I shows mean plasma noradrenaline and plasma adrenaline as well as other pertinent data of the control subjects and the hypertensive patients

In the controls there was a strong correlation between plasma noradrenaline (NA) and age but no difference between females and males The equations of the regression lines are

Females log(NA (ng/ml)×100)=0 90+0 011×(age) $p<0\ 005$

Males $\log(NA\ (ng/ml) \times 100) = 0.81 + 0.013 \times (age)$
$p<0.001$

Systolic and diastolic BPs were also correlated to age ($p<0.01$ 0.05) but not with the plasma noradrenaline concentration Plasma noradrenaline and plasma adrenaline were not correlated with each other

Systolic and diastolic BPs were considerably elevated in the hypertensive patients in comparison with the control subjects ($p<0.001$ 0.001)

Plasma noradrenaline concentration was the same in the hypertensive patients and in the controls (Table I) Plasma adrenaline in the hypertensive females did not differ significantly from the corresponding values in the control females

Plasma noradrenaline increased with age in both female and male hypertensive patients the correlation being significant only in the male subjects ($p<0.05$)

The relationship between plasma noradrenaline and age was the same in the controls and in the hypertensive patients The equations of the regression lines are

All controls
$\log(NA\ (ng/ml) \times 100) = 0.84 + 0.012 \times (age)$
$p<0.001$

All hypertensives
$\log(NA\ (ng/ml) \times 100) = 0.93 + 0.012 \times (age)$
$p<0.005$

The individual data are presented in Fig 1

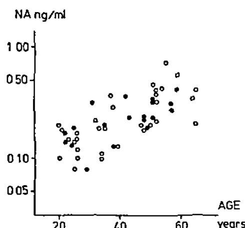


Fig 1 Plasma noradrenaline concentration (NA) obtained in the basal state in hypertensive patients (●) and controls (O) plotted on the logarithmic scale versus age

Table II *Plasma noradrenaline and plasma adrenaline concentrations and blood pressures in 10 hypertensive patients studied before and after treatment with alprenolol and after combined treatment with alprenolol plus hydralazine*

	Untreated	Alprenolol	Alprenolol +hydralazine
BP (mmHg)			
Mean	199/125	181/119	163/108
±S D	17/4	14/7	24/13
Plasma noradrenaline (ng/ml)			
Mean	0.279	0.233	0.344
±S D	0.089	0.067	0.120
Plasma adrenaline (ng/ml)			
Mean	0.027	0.031	0.018
±S D	0.011	0.015	0.011

In the hypertensive patients systolic and diastolic BPs were correlated with age as expected ($p<0.001$ 0.05) There was also a correlation between plasma noradrenaline and systolic BP ($p<0.01$) and between plasma noradrenaline and diastolic BP ($p<0.05$)

Multiple regression analysis was carried out using the plasma noradrenaline concentration as the dependent variable and age and the systolic or diastolic BP as the two independent variables The partial regression coefficient between plasma noradrenaline and age was significant in both cases ($p<0.05$ 0.02) while no significant relationship was obtained between plasma noradrenaline and the BPs

Using systolic or diastolic BP as the dependent variable and age and plasma noradrenaline as the two independent variables the partial regression coefficient between systolic BP and age became significant ($p<0.01$) while no significant relationship was obtained between systolic or diastolic BP and plasma noradrenaline

Table II shows mean values of plasma noradrenaline and plasma adrenaline as well as mean BPs in 10 hypertensive patients who were reexamined after 4 weeks of treatment with alprenolol as well as after 4 weeks of treatment with alprenolol and hydralazine The BP averaged 199/125 mmHg in untreated conditions and had declined to 181/119 mmHg after treatment with alprenolol ($p<0.005$ 0.05) After the combined treatment with alprenolol and hydralazine a further reduction to 163/108 mmHg was obtained ($p<0.05$ 0.005)

There was no change in plasma noradrenaline

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Table III *Urinary excretion of noradrenaline (NA) adrenaline (A) and dopamine (DA) (ng/mg creatinine) in 10 controls and 10 untreated hypertensive patients*

Subj no	Controls NA	A	DA	Hypertensives NA	A	DA
1	26	7	195	36	3	290
2	17	3	132	38	3	152
3	25	7	192	25	8	243
4	41	9	455	21	1	139
5	26	5	170	36	1	133
6	48	8	261	18	4	161
7	14	10	119	18	2	225
8	41	10	181	13	4	157
9	39	7	238	25	8	258
10	32	3	277	20	3	152
Mean	31	7	222	25	4	191

and plasma adrenaline concentrations after treatment with alprenolol while the combined treatment with alprenolol and hydralazine was followed by a significant rise in plasma noradrenaline concentration ($p<0.02$)

Table III shows 24 hour urinary excretion of noradrenaline adrenaline and dopamine expressed in ng/mg creatinine in 10 untreated hypertensive patients and 10 controls Urinary excretion of noradrenaline and dopamine was similar in the two groups while urinary excretion of adrenaline was significantly reduced in the hypertensive patients <0.05)

Table IV shows mean 24 hour urinary excretion f noradrenaline adrenaline and dopamine in 10 hypertensive patients reexamined 4 weeks after treatment with alprenolol Urinary excretion of the catecholamines did not change during treatment with alprenolol

DISCUSSION

The present study demonstrates normal values of noradrenaline and adrenaline in plasma in a group of patients with essential hypertension Urinary excretion of noradrenaline was also similar in the hypertensives and the controls while urinary excretion of adrenaline was reduced in the hypertensive patients These results are in accordance with a number of earlier studies of urinary excretion of catecholamines in hypertensives (2 8 11 14 17) and with our previously published data of total plasma catecholamine concentration in smaller groups of patients with renal and essential hypertension (3)

Plasma noradrenaline measured in the resting state in the supine position is age dependent and this was the case in both controls and hypertensive patients It is not clear whether the rise in plasma noradrenaline with age is due to an increase in adrenergic activity or caused by a decrease in the inactivation of the catecholamines This question deserves further investigation

Plasma noradrenaline correlated with the BP in the hypertensive patients The results of the regression analysis suggest however that this correlation may be explained by the fact that both BP and plasma noradrenaline were age-dependent

Our results indicate that the activity of the adrenergic system is normal in most patients with essential hypertension Despite the high BP plasma noradrenaline was not reduced in the hypertensive patients to values found in patients with neuropathy and in spinal man (4 7)

Our results are contrary to those of Engelman et al (12) DeQuattro and Chan (9) and Louis et al (15 16) who all reported elevated plasma noradrenaline concentrations in patients with essential hypertension The reason for this discrepancy is not clear It should be emphasized that Engelman et al did not mention the age of their patients and although DeQuattro and Chan and Louis et al mentioned the age range this factor was not taken into account in the analysis or mentioned any further

A double isotope derivative technique was used for measurement of noradrenaline and adrenaline in the present study as well as in the studies mentioned above This method is more precise and sensitive than the older fluorimetric methods It is a somewhat complicated and difficult technique to

Table IV *Urinary excretion of noradrenaline (NA) adrenaline (A) and dopamine (DA) (ng/mg creatinine) in 10 hypertensive patients studied before and after treatment with alprenolol*

	NA	A	DA
Untreated			
Mean	25	4	191
±S D	9	2	57
After alprenolol			
Mean	27	3	217
±S D	9	2	39

handle The validity of our modification of this technique has been demonstrated in several previ ous studies and the difference in plasma nor adrenaline concentration between hypertensives and controls reported by the authors mentioned above could easily have been demonstrated in our laboratory if it had existed

Dopamine concentration in the urine is approxi mately 7 times higher than that of noradrenaline Dopamine in the urine is probably derived from the adrenergic system and excreted by tubular secre tion (7) A reduced dopamine concentration in the urine in hypertensive patients has previously been reported by Januszewicz et al (13) and Serrano et al (19) while DeQuattro and Miura (10) found normal values No difference was observed be tween hypertensive patients and control subjects in the present study

Treatment with a β adrenergic blocking agent did not modify plasma noradrenaline and adrenaline in the basal state or urinary excretion of the catecholamines while the combined treatment with alprenolol and hydralazine was followed by a significant rise in plasma noradrenaline This may represent a compensatory increase in adrenergic activity in response to the lowered BP despite the fact that the mean BP was considerably above normal levels in the treated hypertensive patients

It is conceivable that treatment with the β adrenergic blocking agent alone did not modify the plasma catecholamine concentration in the basal state because the decline in BP was very modest

In other experiments (forthcoming publication by Galbo Trap Jensen Christensen) we have meas ured circulating catecholamines in the standing position and during exercise before and after β adrenergic blockade We found the concentra tions of circulating catecholamines to be consider ably higher during the blockade

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Effect of Alprenolol and Hydralazine on Plasma Renin Concentration in Patients with Arterial Hypertension

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ABSTRACT The effect of alprenolol alone and in combination with hydralazine on plasma renin concentration has been studied in 25 patients with arterial hypertension Alprenolol caused a significant fall in plasma renin concentration and in BP but the two falls were not intercorrelated Addition of hydralazine to the treatment regimen produced a further reduction of BP Changes in plasma renin concentration varied greatly during combination therapy A modest, non significant increase in plasma renin concentration was measured compared with the level after treatment with alprenolol It is concluded that mechanisms other than suppression of plasma renin concentration are important for the reduction of BP during β-adrenergic blockade and that the plasma renin concentration probably has no clinical significance with regard to forecasting the antihypertensive effect of alprenolol Even though a significant correlation was found between the fall in plasma renin concentration and the fall in diastolic BP during combination therapy it is uncertain whether changes in the renin–angiotensin system are of primary importance for BP regulation during this therapy

Even though the antihypertensive effect of β adrenergic blocking agents has been well documented the mode of action of alprenolol and other agents in this group is still unclear It is supposed that the reduction of BP is due to an effect on the renin–angiotensin system (3 4) Stimulation of the adrenergic nervous system increases the liberation of renin (7 13) whereas β-adrenergic blockade suppresses its release (1) In some studies (3 4) a correlation has been demonstrated between the antihypertensive effect and the renin suppressing effect of β adrenergic blockade in patients with arterial hypertension Other studies have not been able to collaborate this relationship (2 7)

The studies of Buhler et al (3) suggest that plasma renin activity may be of value as a clinical parameter in the selection of patients best suited for antihypertensive treatment with β adrenergic blockade Beta adrenergic blocking agents would thus be predominantly effective in patients with a high plasma renin level and less suitable for patients with low plasma renin

The purpose of the present study was to investigate the effect of alprenolol on plasma renin concentration Alprenolol was used both alone and in combination with hydralazine In contrast to previous studies employing measurements of plasma renin activity the renin content in plasma in the present study was measured as the plasma renin concentration using a standard human renin as reference

MATERIAL AND METHODS

Patients

Twenty five patients were included in the study 16 men and 9 women with an average age of 42 years (range 24–66) Clinical data are summarized in Table I

Fourteen patients had essential hypertension Two had renovascular hypertension Seven had hypertension in association with a primary renal disease verified by biopsy three had chronic pyelonephritis and four chronic glomerulonephritis Two patients had arterial hypertension after renal allotransplantation In both these patients bilateral nephrectomy had been performed earlier

Table I *Clinical data*

LVH=left ventricular hypertrophy EH=essential hyper tension CP=chronic pyelonephritis CG=chronic glo merulonephritis PT=posttransplantation hypertension RV=renovascular hypertension

Pat no	Sex	Age (y)	Changes in optic fundi	LVH or strain pattern	Creatinine clearance (ml/min)	Etiol ogy
1	♂	59	1	0	83	EH
2	♀	66	2	0	85	EH
3	♀	44	2	0	81	EH
4	♂	33	2	0	120	EH
5	♂	56	2	0	74	EH
6	♂	26	1	0	135	EH
7	♀	50	2	+	65	EH
8	♂	50	2	+	78	EH
9	♂	23	1	0	120	EH
10	♂	28	2	0	132	EH
11	♀	61	2	0	67	EH
12	♂	40	3	+	90	EH
13	♀	51	3	+	33	EH
14	♂	56	2	0	100	EH
15	♀	36	3	0	92	RV
16	♀	45	2	+	62	RV
17	♂	64	2	+	25	CP
18	♂	29	3	0	26	CG
19	♂	27	1	+	6	CP
20	♂	24	2	+	15	CG
21	♀	39	2	+	18	CP
22	♂	24	1	0	100	CG
23	♂	53	2	+	85	CG
24	♀	36	3	0	65	PT
25	♂	26	1	+	91	PT

* Grading of Keith Wagener

The hypertensive organic lesions included 1st degree retinal changes in 6 patients 2nd degree in 14 and 3rd degree in 5 (Keith Wagener) On ECG hypertrophy or strain was present in 11 patients and 9 had an increased cardio/thoracic ratio Patients with essential and renovascular hypertension had a creatinine clearance of 33–135 *ml/min and one patient (no 13) had proteinuria* 0 5 g/day Among patients with renal disease two had normal renal function with a creatinine clearance of 85–100 ml/min whereas five had various degrees of renal insufficiency with a creatinine clearance of 6–26 ml/min The two patients with posttransplantation hypertension had stable graft function during the period of the study with a creatinine clearance of 60–70 and 85–95 ml/min

Hypertension in 12 patients had been known for less than a year in 5 between 1 and 5 years and in 8 between 6 and 15 years Thirteen patients had not previously re ceived antihypertensive therapy The remaining 12 pa tients had received α methyldopa and/or diuretics previ ously

Procedure

All patients underwent 2 or 3 periods of study each period lasting 4 weeks During the first or control period all drugs were discontinued In the second period after a 4 week pause in treatment alprenolol was administered (Aptin® durettes) 400–1200 mg/day in 2–3 divided daily doses In 21 patients BP was unacceptable at the end of the second period and alprenolol was supplemented with hydralazine (Apresolin®) 75–225 mg/day in 3 divided doses Patients were ambulant and there were no dietary restrictions with regard to salt At the end of each period BP was measured after at least one hour s rest in the supine position

METHOD

Plasma renin concentration was measured using the method described by Giese et al (6) This involves radioimmunologic measurement of angiotensin I after previous dialysis of plasma incubation at 37°C and pH 7 4 both with and without the addition of a human standard and extraction of angiotensin I produced Plasma renin con centrations are given in Goldblatt units (GU) using the *above mentioned human renin as reference Plasma renin* concentrations were measured between 8 and 9 a m in 15 normal individuals on liberal salt intake after at least one hour s rest in the supine position and after 8 hours fasting In these individuals the mean value was 36 μGU/ml (range 10–79) The coefficient of variation for analysis (day to day) was 11% Blood samples for renin analysis in pa tients in this study were taken between 8 and 9 a m after the patients had fasted from midnight the previous day and had rested in the supine position for an ho r

Dr J Giese Department of Clinical Physiology Amts sygehuset Glostrup Denmark provided antiserum against angiotensin I and the Medical Research Council Division of Biological Standards National Institute for Medical Research Mill Hill London placed Renin Human 68/356 at our disposal

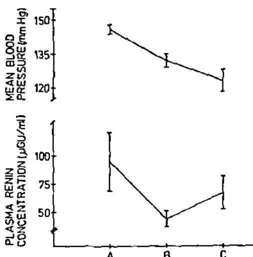


Fig 1 BP and plasma renin concentration (means ±S E M) before treatment (*A*) during treatment with alprenolol (*B*) and during combined treatment with al prenolol and hydralazine (*C*)

Table II *Blood pressure (mmHg) before treatment (A) during treatment with alprenolol (B) and during the combined treatment with alprenolol and hydralazine (C)*

Pat no	A	B	C
1	170/115	160/100	150/85
2	190/115	160/105	160/100
3	220/120	200/110	165/105
4	220/130	170/110	170/115
5	225/125	190/115	180/100
6	170/120	130/105	125/85
7	240/140	220/125	155/95
8	180/130	150/105	120/90
9	190/110	175/120	150/105
10	150/110	130/90	–
11	210/130	220/120	200/115
12	180/140	150/115	170/80
13	210/120	200/130	200/120
14	270/125	220/125	150/95
15	190/120	170/110	200/110
16	190/125	210/140	210/145
17	220/125	160/95	–
18	170/130	160/120	170/115
19	170/130	140/100	–
20	180/140	160/110	160/90
21	210/135	200/135	210/135
22	150/115	130/100	–
23	180/125	180/130	140/100
24	170/110	160/100	150/95
25	180/115	150/105	125/70
Average	191/124	172/113	162/102
S E M	4 9/1 8	5 7/2 6	6 3/3 9

RESULTS

Blood pressure

The effect of alprenolol and hydralazine on BP is given in Table II and Fig 1 Alprenolol induced a significant fall in BP from 191/124 to 172/113 mmHg ($p<0\ 01/0\ 01$) In 21 patients the addition of hydralazine to alprenolol regimen produced an additional significant fall in BP to 162/102 mmHg ($p<0\ 01/0\ 01$) In 9 patients the diastolic BP was higher than 100 mmHg at the end of the study

Plasma renin concentration

Table III and Fig 1 show the plasma renin concentrations before and after antihypertensive treatment Of the 14 patients with essential hypertension 11 had a normal plasma renin concentration Three of these 11 patients (nos 2 5 11) had low concentrations within the normal range 10–15 μGU/ml In the remaining 3 patients with essential hypertension the plasma renin concentration was high 218–493 μGU/ml

One of the patients with renovascular hypertension (no 15) had a clearly increased plasma renin concentration of 126 μGU/ml whereas the other (no 16) had a normal plasma renin concentration of 47 μGU/ml

Two of the patients with primary renal disease (nos 20 23) had high plasma renin concentrations whereas the others in this group had a normal plasma renin concentration as did the two post transplant patients with hypertension

After alprenolol treatment there was a statistically significant fall in the plasma renin concentration from 95 to 47 μGU/ml ($p<0\ 01$) There was no significant difference between the plasma renin concentrations before and after combination treatment the mean values being 95 and 67 μGU/ml ($p>0\ 05$) respectively Even though the mean plasma renin concentration increased from 47 after alprenolol to 67 μGU/ml after combination treatment this difference was not significant

Table III *Plasma renin concentration (μGU/ml) before treatment (A) during treatment with alprenolol (B) and during the combined treatment with alprenolol and hydralazine (C)*

Pat no	A	B	C
1	24	20	29
2	10	11	9
3	38	29	28
4	48	24	40
5	15	20	10
6	39	23	18
7	34	20	9
8	48	30	25
9	51	51	53
10	45	48	–
11	15	11	15
12	493	94	89
13	218	64	139
14	475	145	210
15	126	91	205
16	47	58	63
17	63	18	–
18	42	38	70
19	30	16	–
20	255	114	152
21	51	49	50
22	33	25	–
23	84	107	114
24	46	40	39
25	38	33	44
Average	95	47	67
S E M	26	7 2	14

Relationship between blood pressure and plasma renin concentration

The fall in systolic as well as in diastolic BP during alprenolol treatment alone was not correlated to the fall in plasma renin concentration On the other hand a statistically significant correlation was found between the fall in diastolic BP ($p<0.05$) but not in systolic BP ($p>0.05$) after combination treatment compared with the levels before treatment

In 6 patients with high plasma renin concentrations (84–493 μGU/ml) mean BP was reduced from 193/128 to 180/120 mmHg after β adrenergic blockade and to 161/99 mmHg after combination treatment The corresponding values for the 3 patients with low plasma renin concentrations (10–15 μGU/ml) were 208/123 before treatment 190/113 after alprenolol treatment and 180/105 mmHg after combination therapy

DISCUSSION

The present results like previous studies (5 10 11 14) indicate that alprenolol as well as a combination of alprenolol and hydralazine induced a significant reduction of BP in patients with arterial hypertension The reason why BP was reduced to a lesser degree in our study than in those mentioned above may have been due to differences in the degree of hypertension since the treatment regimens did not differ markedly

The method used in this study for measurement of plasma renin concentration was independent of the plasma content of renin substrate and of activators and inhibitors of the renin–angiotensin system This is in contrast with previously published measurements of plasma renin activity Thus plasma renin concentrations in this study were solely an expression of the renin content of plasma and any effect by the antihypertensive agents on renin substrate or other factors which might affect the renin–angiotensin system does not need to be considered Our study shows that alprenolol causes a significant reduction of plasma renin concentration This agrees well with what other investigators have found i e alprenolol (4) and propranolol (3 7) cause a significant reduction of plasma renin activity It has therefore been suggested that the antihypertensive effect of β adrenergic blocking agents is related to a suppression of the renin–angiotensin system This suggestion is supported by the studies of Buhler et al (3) and of Castenfors et al (4) showing that the fall in BP was correlated to the plasma renin reduction during propranolol or alprenolol therapy This is in contrast to the results of Hansson et al (7) and to our results The lack of agreement is difficult to explain but may be related to differences in the etiology and degree of severity of the hypertension in the various studies It does however appear more reasonable to suggest that mechanisms other than suppression of the renin–angiotensin system contribute to the antihypertensive effect of alprenolol Some investigators (9) have suggested that the antihypertensive effect of β adrenergic blocking agents is primarily related to a reduction of the cardiac output and a secondary resetting of the baroreceptors to a lower BP level

Previous studies suggest that hydralazine stimulates renin release (12) Our studies show that changes in plasma renin concentration during combination therapy with alprenolol and hydralazine vary greatly There was a correlation between the fall in plasma renin concentration and the fall in diastolic but not systolic BP after combination therapy This suggests a relationship between the effect on the renin–angiotensin system and changes in BP during combination therapy On the basis of our studies however it is not possible to determine whether this relationship is of primary significance for the BP reduction The significance of changes in the renin–angiotensin system during treatment with β adrenergic blocking agents and vasodilatators in combination requires further study

Buhler et al (3) have found that the antihypertensive effect of propranolol is markedly better in patients with a high plasma renin activity Our studies were not able to confirm this finding with regard to alprenolol In 6 patients with high plasma renin concentrations BP was reduced to approximately the same level as in patients with low plasma renin concentrations Even though our study group was small we consider it uncertain whether plasma renin has any practical clinical relevance with regard to forecasting the antihypertensive effect of β adrenergic blocking agents

ACKNOWLEDGEMENTS

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Diazoxide in the Management of Severe Hypertension

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BSTRACT Fourteen patients with severe hyper nsion have been given i v diazoxide in a dosage f 5 mg/kg b wt The material comprised 2 patients ith malignant nephrosclerosis, 4 with chronic ephropathy and severe reduction of renal func on, 1 patient with chronic pyelonephritis, 1 with enovascular hypertension and 6 patients with ssential hypertension in malignant phase All pa ents attained a controllable blood pressure Eight atients needed only one injection, while the remain ng patients required 2–5 injections, and concomitant urosemide therapy The retinopathy improved in most patients and renal function was unchanged in he azotemic patients No serious adverse effects were een except one hypotensive episode Diazoxide is asy to handle dosage can be predetermined, monitoring is simple and we find diazoxide to be a aluable drug in severe hypertension

Although several hypotensive agents are available n the management of severe hypertension the reatment is often insufficient to avoid progressive enal insufficiency or other tissue damage Patients with sustained hypertension usually have a normal ardiac output and a raised peripheral vascular esistance It seems logical to reverse this situation y using a drug which dilates the vascular smooth muscle of the systemic arterioles specifically (7) o accomplish this three drugs (dihydralazine hlorpromazine trimethaphan) have been used All ave drawbacks in the management of acute ypertensive crisis Dihydralazine (Nepresol®) has direct relaxing action on arteriolar smooth muscle ut the dosage varies immensely making treatment ifficult to monitor Usage of the α blocking activ ty of chlorpromazine is hampered by the concomi ant actions on cerebral and myocardial function while trimethaphan (Arfonad®)—a ganglion blocking agent—relaxes both resistance and capacitance vessels and may depress myocardial function (7) Diazoxide—a non diuretic benzothiadiazine deriva tive acting specifically on the smooth muscle in peripheral arterioles—is mentioned by Koch Weser (7) as an excellent and logical alternative

MATERIAL AND METHODS

In a 1 year period the efficiency of diazoxide was eval uated in 14 consecutive patients with severe hyperten sion 10 males 4 females mean age 60 years (range 27–77) Included in the study were patients with a diastolic BP above 125 mmHg and either severe fundu scopic changes or acute congestive heart failure BP was followed every 15 min in the recumbent position for at least one hour or until it had stabilized

Table I shows pretreatment clinical data for the materi al Six patients had severe reductions in renal function (creatinine clearance 3 5–25 0 ml/min) Renal biopsy documented malignant nephrosclerosis in 2 patients (nos 5 and 12) and chronic glomerulonephritis in 1 patient (no 14) while patients 6 7 and 8 had end stage kidneys Changes suggestive of chronic pyelonephritis were found in 1 patient (no 2) with slight reduction in renal function and renovascular hypertension was documented in another patient (no 13) with normal renal function The remaining 6 patients were considered to have essential hypertension based on history and renal function tests Pheochromocytoma was excluded in suspected cases

Rapid i v injections of diazoxide (5 mg/kg b wt) were given within 30 sec A positive response was defined as a significant decrease in diastolic BP If the diastolic BP later increased to above 125 mmHg diazoxide was repeated in the same dosage as needed As soon as possible oral treatment with a combination of hydralazine (Apresolin®) and propranolol (Inderal®) was instituted unless contraindications were present Repeated injec tions of diazoxide were preceded by furosemide (Lasix®)

Before administration of diazoxide ophthalmoscopy and ECG were performed Venous blood samples were drawn for measurements of urea creatinine sodium potassium and glucose repeated 24 hours later and when

Table I *Pretreatment clinical data for 14 patients with severe hypertension*

Pat no	Sex	Age (y)	Initial BP (mmHg)	Eye ground findings	Hypertensive encephalopathy	Other complications	Duration of hypertension (y)
1	♀	74	260/140	F H II	–	–	8
2	♂	58	200/130	F H II	–	–	6
3	♀	77	230/160	?	+	–	5
4	♂	73	240/130	F H III	–	–	7
5	♂	27	200/140	F H IV	+	Acute renal failure	Onset
6	♀	63	220/130	F H III	+	–	>5
7	♀	64	270/150	F H III	–	Acute pulm edema	30
8	♂	66	220/130	F H III	–	–	5
9	♂	61	260/160	F H IV	+	Cerebral hemorrhage	Onset
10	♂	67	270/140	F H III	+	–	Onset
11	♂	56	270/150	F H III	–	Acute pulm edema	2
12	♂	56	240/140	F H IV	+	Acute renal failure	Onset
13	♂	44	220/150	F H III	+	–	4
14	♂	49	220/140	F H III	–	–	6

indicated Both ECG and ophthalmoscopy were performed daily during the first week In the 6 patients with severe reduction in renal function natriuresis and creatinine clearance were followed during the hospitalization At gross hypotension (defined as a systolic BP below 100 mmHg) metaraminol (Aramin®) was given (5 mg i v) After discharge all patients were controlled every 3rd week in the Out patient Clinic

RESULTS

Blood pressure Positive responses were obtained in all 14 patients The typical response is outlined in Fig 1 As will be seen from Table II the maximal response was elicited within the first 5 min after injection A plateau was reached after an average of 16 min (range 10–35) After the initial dose BP remained controllable with concomitant oral therapy in 8 patients In 6 patients repeated doses of diazoxide were required during the first 24–96 hours From the figures given in Table III it seems as if patients with severe renal insufficiency require repeated injections of diazoxide Long term results have been satisfactory in all patients as BP remained under control (observation period 3–12 months)

Resetting of baroreceptors probably was seen in 2 patients as the dose of propranolol could be reduced from 1 000 to 160 mg daily in patient 14 and patient 13 remained normotensive for 2 months without antihypertensive medication following one injection of diazoxide

Eye ground Eleven patients had severe fundu

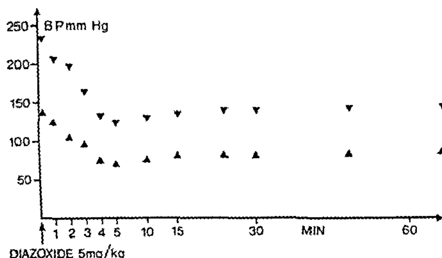


Fig 1 Typical response of blood pressure during treatment with diazoxide

Table II *Systolic and diastolic blood pressure in 14 patients before and 5 15 and 30 min after iv injection of diazoxide 5 mg/kg b wt (mean ±S D)*

BP (mmHg)	Pretreatment level	After diazoxide 5 min	15 min	30 min
Systolic	237±25	144±34	160±33	165±27
Diastolic	142±11	87±11	91±14	92±13

scopic changes (grade IV in 3 patients and grade III in 8) prior to diazoxide In all these patients improvement of the eye ground findings to grade II or less was observed in 9 of them within 4–5 days No change was observed in patients with grade II eye ground findings

ECG No significant changes were observed during the first week although 2 patients (nos 5 and 11) developed increasingly inverted T waves in left sided precordial leads

Blood glucose concentration Twelve patients had normal pretreatment values without significant increases (mean increase 4%) 24 hours after diazoxide injection Patient 14 exhibited a temporary increase from 85 to 156 mg/100 ml and patient 9 with cerebral hemorrhage had an increase from 210 to 285 mg/100 ml Antidiabetic treatment was not given to any of the patients

Renal function The 6 azotemic patients will be considered with particular interest Creatinine clearance before diazoxide injection and one week later is shown in Table IV Significant changes were not seen

Fluid and electrolytes No changes in serum concentrations of sodium potassium and bicarbonate were observed The natriuresis cannot be evaluated as changes in the ingestion of sodium took place simultaneously

Adverse effects Burning pain from the used vein is frequently observed We have not observed nausea vomiting flushing or headache In patient 2 systolic BP fell to 60 mmHg 5 min after injection of diazoxide I v metaraminol instantly elevated the BP to pretreatment levels followed by a gradual decrease to 120/80 mmHg Clinical history RBBB for 2 weeks and subsequent angina suggested a 7 days old myocardial infarction

Causes of death Patient 8 died during the hospitalization from low output heart failure and autopsy revealed several old myocardial infarctions As daily measurements of body weight and electrolytes were not significantly changed sodium retention is excluded

Table III *Number of diazoxide injections in 14 patients related to renal function as judged by creatinine clearance*

Creatinine clearance (ml/min)	No of injections 1	2	3	>3
>50	6	2	0	0
25–50	1	0	0	0
<25	1	1	2	1
No of pats	8	3	2	1

DISCUSSION

The use of a standard 300 mg i v dose of diazoxide in severe hypertension has been stressed by several workers Finnerty et al (5) and Beamer and McDonald (1) have gained control of BP in 29 of 33 patients and in 6 of 10 patients respectively From a pilot study (2) we have decided to use an individual dosage regimen of 5 mg/kg b wt All patients responded positively Furosemide was given to avoid the well known sodium retaining and antidiuretic actions (12) of diazoxide when repeated doses were needed In accordance with Finnerty (3) we have not observed resistance to treatment Sellers and Koch Weser (10) stress that the efficiency of diazoxide is dependent on the rate of injection caused by the high degree of protein binding Its rapidity of effect gives diazoxide a distinct advantage over dihydralazine infusion

Table IV *Creatinine clearance in 6 hypertensive patients with renal insufficiency before treatment with diazoxide and one week later*

When significant changes occurred after 3 months the results are given within parentheses

Pat no	Diagnosis	Creatinine clearance (ml/min) Before diazoxide	After diazoxide
5	Malignant nephrosclerosis	8 0	6 0 (17 0)
6	Chronic nephropathy	3 5	3 5
7	Chronic nephropathy	3 5	4 0
8	Chronic nephropathy	9 5	9 0
12	Malignant nephrosclerosis	9 5	7 0 (2 5)
14	Chronic glomerulonephritis	25 0	27 0

Controversial opinions exist regarding the regression of the funduscopic changes following i v diazoxide (1 9) Regression of grade III and IV changes was seen invariably in our material after a few days Angiotensin II seems to be the culprit in hypertensive vascular damage (6) A possible explanation of the salutary effects on the eye ground in our patients could be either abolition of the peripheral actions of angiotensin II by diazoxide or inhibition of renin secretion by the concomitant propranolol treatment

When ECG changes are seen after diazoxide the most constant findings are increased left ventricular hypertrophy and strain and signs of subendocardial ischemia (1 4) These findings could be related to increased sensitivity of baroreceptors The phenomenon of resetting of baroreceptors is stressed by Finnerty et al (4) who used repeated injections of diazoxide in 16 patients unresponsive to oral therapy and eventually modified the hypertensive diesease in 14 of them In at least 2 of our patients we have found evidence of resetting of baroreceptors

As i v diazoxide is known to produce slight hyperglycemia (11) we have not investigated further into this subject The present material and that of Mroczek et al (9) convincingly prove that it is possible to reduce BP in patients with severe renal insufficiency without provoking a reduction in ...inine clearance Patient 12 with malignant ...osclerosis who remained on regular dialysis ...eatment belongs to the group of patients in whom bilateral nephrectomy usually is necessary to obtain control of the BP Before performing bilateral nephrectomy in patients with life threatening *hypertension a trial with diazoxide is mandatory*

Adverse effects were infrequent and usually of no significance However it should be pointed out that diazoxide should be used with caution in patients with suspected myocardial infarction

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A 3-year Follow-up of Middle-aged Men with Borderline Blood Pressure

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ABSTRACT Ninety-eight middle-aged men with untreated borderline raised blood pressure (BP) have been reexamined after three years Twenty five subjects were considered to be hypertensive motivating a shorter interval for rescreening Systolic BP had decreased in 23%, diastolic BP in 46% and both pressures in 15% Those who developed hypertension had higher systolic BP at the initial examination A family history of hypertension was more common in this group

Data from 26 insurance companies collected by Metropolitan Life (1) show that mortality increases with increasing systolic and diastolic blood pressure (BP) The beneficial effect of treating high BP has been shown in controlled studies (32 33) However the limit at which treatment should be started is not definite It is therefore of importance to follow subjects with BP below the chosen limit One reason is to follow the BP in order to select those subjects who develop a rise in BP requiring treatment as well as to establish the optimal interval for reexamination Another reason is to evaluate the possible occurrence of hypertensive complications below this limit This is of particular interest in subjects with borderline blood pressure (BBP)

In the present study a group of middle aged men with BBP has been reexamined after three years The aim was to assess the incidence of hypertension appearing over this period as well as to identify any factors predisposing to this development

MATERIAL

This was a substudy from a health examination survey in the City of Uppsala (10) Ninety-eight men aged 50 were *recruited from subjects with untreated supine BP of* 160–175/100 and/or ≥160/100 mmHg in sitting or standing position but not fulfilling the criteria for antihypertensive treatment supine diastolic BP (DBP) ≥105 mmHg (11)

The BP was measured in supine and in standing position in 41 men In 57 men it was measured in supine and sitting positions Most subjects (46 9%) were recruited because of elevated DBP in standing or sitting position Seventy four individuals had DBP ≥100 mmHg in one of these positions Only one subject was included due to supine *DBP=100 mmHg as the sole criterion The 98 men* corresponded to a participation rate of 88 3% One subject who had an aortic insufficiency is not included in the calculations below

METHODS

The initial examination and the reexamination were performed under similar conditions The subjects were asked to fast and to refrain from smoking since the preceding midnight The examination started at 7 30 a m and the BP was measured by the same registered nurse on both occasions Each individual had his two examinations in the same season of the year

The BP was measured on the right arm after lying prone for 10 min and after 2 min sitting and standing Mercury manometers (Kifa Ercameter wall model) were used The BP cuff had a rubber bladder 12 5×35 cm Systolic BP (SBP) and DBP were read to the nearest 5 mmHg mark *The DBP was measured at the disappearance* of the Korotkoff sounds (phase 5) At the reexamination a supine BP of ≥175/105 mmHg was defined as hypertension The systolic figure was estimated from the WHO recommendation (35) that BP ≥160/95 mmHg should be the lower limit for hypertension in epidemiologic work using the formula

$$\frac{\text{SBP to be determined}}{160} = \frac{105}{95}$$

(Only the man mentioned above with aortic insufficiency had supine SBP of 175 mmHg at the initial examination)

The family history of cardiovascular disease and hypertension was obtained by a self administered questionnaire *ad modum Collen et al* (4) This was also used to get information about physical activity at work and during

Table I *Clinical and laboratory data in 58 men initially and after 3 years*

Men with antihypertensive lipid-lowering and antidiabetic therapy not included

	Initial examination		After 3 years		Statistical analyses	
	Mean	*S D*	*Mean*	*S D*	*t value*	*Significance*
Weight index[a]	1 15	0 13	1 14	0 13	0 32	n s
Pulse rate/min	72 7	12 6	70 2	10 4	1 13	n s
Serum cholesterol (mg/100 ml)	222	33	218	31	0 71	n s
Serum triglyceride (mmol/l)	1 54	0 84	1 45	0 74	0 65	n s
Serum triglyceride log (mmol/l×100)	2 1353	0 2116	2 1150	0 1953	0 54	n s
Serum uric acid[b] (mg/100 ml)	4 37	0 83	4 70	1 22	1 66	n s
Serum creatinine[b] (mg/100 ml)	0 95	0 16	0 90	0 16	1 55	n s

[a] Actual/ideal weight (18) [b] Missing data on one subject
n s =non significant

leisure stress experience and smoking habits The classification has been presented elsewhere (10)

The laboratory analyses at both examinations were performed by the methods used routinely in the Department of Clinical Chemistry University Hospital Uppsala and have been presented elsewhere (10) The calculation of the ideal weight was done using the tables of Lindeberg et al (18)

Conventional statistical methods were used for calculation of mean value and S D Significance of differences between mean values was estimated with Student s *t* test (2-tailed test) In non-parametric distributions the rank sum test was used Differences in frequencies were tested means of the χ^2 test with Yate s correction The paired was used for analysing the differences of paired ervations Regression analyses were performed by the of least squares The accepted level of significance was $p<0\ 05$

RESULTS

Antihypertensive therapy had been instituted in 11 men between the two examinations Another 20 men had received treatment for hyperlipidaemia and 9 for impaired glucose tolerance The remaining 58 men did not receive any therapy over the 3 year period Their clinical and laboratory data at the two examinations are shown in Table I No significant differences were found

Blood pressure development

The average BPs of the 86 untreated subjects are shown in Table II There was a significant rise in SBP in supine sitting and standing positions The DBPs were not found to be higher at the second examination The changes (≥5 mmHg) in supine BP in the 86 untreated men are shown in Fig 1 Sixty four per cent of the men had higher SBP and 24% higher DBP In 19 subjects both pressures had increased Eight of these were among the 14 men considered to be hypertensive at the reexamination On the other hand 23% of the men had lower SBP

Table II *Mean systolic (SBP) and diastolic (DBP) blood pressures (mmHg) in untreated men initially and after 3 years*

	No of subj	Initial examination		After 3 years		Level of significance
		Mean	S D	Mean	S D	(*p* value)
SBP supine	86	145 7	11 5	152 3	15 7	<0 01
DBP supine	86	93 0	5 8	91 4	8 1	>0 05
SBP sitting	53	149 2	12 0	155 7	15 7	<0 01
DBP sitting	53	99 4	6 3	98 5	7 2	>0 05
SBP standing	33	149 7	12 3	158 8	15 7	<0 05
DBP standing	33	103 1	4 1		8 4	>0 05

[a] Rank sum test

Diastolic Blood Pressure				
	2	0	22	Higher
	5	6	19	Unchanged
	16	7	23	Lower
	Lower	Unchanged	Higher	
	Systolic Blood Pressure			

Fig 1 Changes (≥5 mmHg) in supine blood pressure over a 3 year period in 86 untreated men (%)

and 46% had lower DBP Both pressures had decreased in 13 subjects

Fourteen of the 86 untreated subjects (16 3%) had a BP in excess of the accepted limit of supine BP used to define hypertension (≥175/105 mmHg) after three years The distribution of the initial supine BPs is shown in Fig 2 These 14 men and the 11 who had received therapy prior to the reexamination were found among those with the higher pressures Thus 15 of these 25 men (60%) had SBP ≥155 mmHg which is 55 6% of all the men above that level (the subject with aortic insufficiency not included) The DBPs of the 25 men were not found among the highest DBPs to the same extent Twenty two of the 25 future hypertensives had the two highest supine SBP (165 and 170 mmHg) and DBP levels (95 and 100 mmHg) Thus 36 1% of the males with initial supine BPs of ≥165/95 mmHg became hypertensives compared to 8 3% of those with BPs below that level

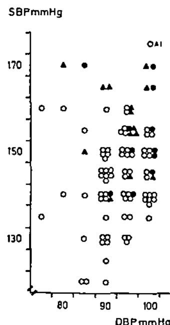


Fig 2 Distribution of supine blood pressure in 98 men at the screening examination AI=aortic insufficiency ●=treated BP after 3 years ▲=untreated BP ≥175/105 mmHg at the reexamination O=the remaining subjects

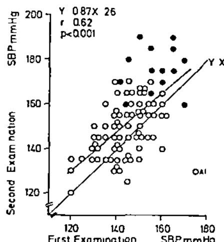


Fig 3 Relation between supine systolic blood pressures (SBP) in 87 untreated men initially and after 3 years AI=aortic insufficiency ●=BP ≥175/105 mmHg O=BP<175/105 mmHg at the 3 year examination

The relationship between untreated supine SBP and DBP at the two examinations is shown in Figs 3 and 4 There was a significant correlation between both SBPs and DBPs For SBP the regression line was above the line $y=x$ This means that the SBPs at the reexamination were higher than initially No such correlation was found for the DBPs

Using the paired observation test the increase in supine SBP between the two examinations was 18 6 mmHg ($p<0\ 001$) in the 14 untreated hypertensives compared to 4 9 mmHg ($p<0\ 001$) in the 72 remaining subjects The corresponding results for the DBP were a rise of 7 5 mmHg ($p<0\ 001$) and a reduction of 3 0 mmHg ($p<0\ 001$) respectively This greater increase in SBP as well as in DBP among the 14 hypertensives was highly significant ($p<0\ 001$) The findings for sitting and standing BPs were similar However the changes in DBP from supine to sitting or from supine to standing positions did not separate the future hypertensives from the remaining subjects

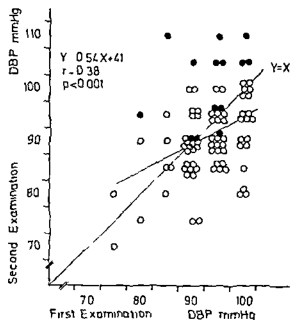


Fig 4 Relation between supine diastolic blood pressures (DBP) in 87 untreated men initially and after 3 years Symbols as in Fig 3

Predictability of blood pressure development

Is there any possibility of detecting future hyper tensives among subjects with BBP?

Table III shows a comparison between the initial BPs as well as some laboratory analyses in the hypertensive group and the rest of the material The mean SBP was significantly higher in the hyperten sive group There were no differences between the other parameters Thus the relative body weight was 1 19 and 1 16 in the two groups respectively

There was no evidence that the hypertensive group had gained more weight over the 3 year period

The resting pulse rates were similar in the hypertensives and the remaining subjects (Table III) However the mean pulse rate in the total BBP group 72 7±11 3 was significantly higher ($p<0\ 001$) than in a population sample from the same study 68 4±10 4 (10) On the other hand the pulse rate in the BBP group was significantly lower ($p<0\ 05$) than in untreated hypertensives 76 7±12 6 (11)

The frequency of some other variables has also been compared between the 25 hypertensives and the rest of the material ($n=72$) A family history of hypertension (as regards father and/or mother) was obtained in 48% of the hypertensives and in 27% of the remaining subjects ($p=0\ 05$) The number of dead parents was similar in both groups More par ents of the hypertensives had died from cardiovascu lar causes (myocardial infarction and cerebrovascu lar disease) The percentages were 48 and 31 re spectively The difference was not significant

A low physical activity at work (predominantly sedentary) was reported by 11 men (44%) in the hypertensive group and by 29% in the remaining material This difference was not significant No differences could be detected in stress experience and smoking habits between the two groups

DISCUSSION

The incidence of hypertension in a BBP group is naturally dependent upon the criteria used for selecting the sample The Hypertension Study

Table III *Initial clinical and laboratory data in 25 future hypertensives compared to the 72 remaining subjects in the borderline blood pressure group*

	Hypertensives[a]		Remaining subjects		Level of significance
	Mean	S D	Mean	S D	(p value)
SBP supine	155 6	10 0	143 6	10 5	<0 001
DBP supine	94 6	5 6	93 0	5 9	>0 05
Pulse rate/min	73 7	13 0	72 3	10 8	>0 05
Weight index	1 19	0 17	1 16	0 15	>0 05
Serum cholesterol (mg/100 ml)	225	41	238	40	>0 05
Serum triglyceride (mmol/l)	2 08	0 97	1 73	1 08	>0 05[b]
Serum uric acid (mg/100 ml)	4 59	1 03	4 36	0 73	>0 05

[a] 14 had developed a supine BP ≥175/105 mmHg over a 3 year period 11 had received therapy
[b] Calculated on logarithm values

Group in the USA (13) has recommended a BP of 140–159/90–94 mmHg for defining borderline hypertension in ages 40 and above These criteria are close to those recommended by the WHO (35) Hypertension is often defined as casual BP ≥160/95 mmHg (7 27) Wilhelmsen et al (34) have used a casual BP of ≥175/115 mmHg for diagnostic work up and treatment These pressures corresponded to 162/101 mmHg as morning cut off points

The pressures used for defining BBP in the present study supine 160–175/100 mmHg were thus similar to those of hypertension in other studies (7 27 34) The criteria used for BPs obtained in sitting and standing positions were ≥160/100 mmHg This was done in order to study whether any of the BPs obtained in these different ways or the BP increment from supine to one of these positions, could predict the future hypertensive The primary selection criteria were sitting or standing DBPs ≥100 mmHg

Several cross sectional as well as longitudinal studies have shown that SBP and DBP both increase with age independently of the increase in body weight with age (3 5 7 31) Miall and Lovell (20) using multiple regression analysis found that this change in BP was more closely related to pressure than to age Therefore it seemed natural in a study of BP development to follow subjects with BBP

In the present study there was a significant rise in supine as well as sitting and standing SBP Furthermore as shown by the regression line i Fig 3 this increase holds true across the whole range of SBPs However there were also subjects who had lower BP after three years For DBP this decrease is demonstrated in Fig 4 One explanation of this may be the statistical phenomenon of regression towards the mean (25) Another factor to consider is the individual variability of BP (19 28)

It is of great practical importance to be aware that in some subjects an elevated BP may decrease over a period This was illustrated by 23% of the subjects who had a reduced SBP and 46% who had a reduced DBP over the 3 year period It is therefore of particular importance to be sure that the recorded BPs are representative for the individual before instituting antihypertensive therapy These aspects have been further discussed in a therapeutical trial of subjects from the same population study (12) In spite of this variability several prospective studies have shown that even a single casual BP has a good long term prognostic significance (16 24 28 29)

The importance of standardized conditions for the examination procedure has been discussed by McKeown et al (19) These authors found no increase in mean BP over a 3 year period They suggested this to be due to higher air temperatures at the time of the second reading In the present study precautions were taken to achieve similar conditions at the two examinations so that a systematic error between the two readings seems unlikely

The great number of subjects who developed hypertension over the three years may be explained by the relatively high initial BP values This agrees well with the findings of Miall and Lovell (20) In this respect the importance of decreased compliance of the aortic wall with aging causing systolic hypertension has to be considered (15 23)

The incidence of hypertension in the BBP group was 25 5% including those men already on therapy This means that at least 2 6% of the total population (n=956) developed hypertension over this 3 year period corresponding to an annual incidence of 0 9% Stamler et al (29) have reported 1% and Berglund et al (2) found 2% Julius and Schork (14) have reviewed the literature on borderline hypertension They concluded that the risk of developing hypertension was at least twice that for normotensives Others have stated the risk to be about four times (17 26)

The 14 untreated hypertensives in the present study showed a significantly greater rise in supine SBP and supine DBP than did the other untreated men Fig 2 shows that the future hypertensives (n=25) had supine BPs in the higher regions of the distribution at the initial examination the SBP being the best predictor This finding was in accordance with Thomas (30) who over an observation period of 8–21 years found that 81% of those who developed hypertension had initial BPs in the upper quintile Miall and Lovell (20) also pointed out that those with a higher initial reading showed a steeper BP rise An analysis of the sitting and the standing BPs gave no further information

Another purpose was to study the possibility of predicting the future hypertensives The role of inheritance has been reviewed by Hamilton et al (8) They found that relatives of hypertensives showed higher pressures in all age groups than relatives of normotensives In the present study the role of inheritance was illustrated by the greater frequency of

a history of hypertension among the parents of the future hypertensives Similar results have previously been reported in hypertensives (8 9)

Many patients with borderline hypertension have a hyperkinetic circulation where the increased cardiac output seems to be due to an increased heart rate (27) The mechanism is not clearly understood A recent report by Ellis and Julius (6) supports the concept that the autonomic nervous system is responsible for the rise in cardiac output heart rate and stroke volume in these patients

Thus increased heart rate has been discussed as a predictor for developing hypertension (2 21 22) The difference between the average initial pulse rate in the 25 future hypertensives compared to that in the remaining 72 subjects was insignificant However the mean pulse rate in the BBP group was *significantly higher than in a population sample of* the same age

No predictability was found when analysing other factors discussed (2 14 29) such as overweight history of weight gain physical activity stress experience and smoking habits This was hardly to be expected in such a small material

This study has shown that in the group of men with BBP there was a significant rise in the mean SBP over a 3 year period and that 25% of them developed hypertension This motivates a shorter interval for rescreening Potential hypertensives were found to have higher initial BP especially SBP and more often a family history of hypertension

As a consequence of this investigation it is desirable to evaluate the BP development of individuals in the lower region of the BP distribution Such a study is under way

ACKNOWLEDGEMENT

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Seventy-year-old People in Gothenburg
A Population Study in an Industrialized Swedish City

I *General Presentation of the Study*

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ABSTRACT The objectives of this cross-sectional population study of 70-year-old people in Gothenburg, Sweden were to make a survey of the social and medical conditions of the population, to obtain basic data for planning the care of the elderly, to contribute to the knowledge of normal ageing processes and of normal criteria within the age group, and to offer the subjects a thorough medical examination A representative systematic sample comprised 1 148 propositi (521 males and 627 females) The study comprised a homecall part regarding basic personal data, dwelling conditions economy, social and physical communications previous migration, educational level, previous and any present professions, need for care, consumption of health care and drugs Observations and measurements were done of illumination at the habitual site of reading and a simple test of visual acuity was performed Later on, examinations at the Out patient Department of the Geriatric Hospital were performed, comprising in all probands general medical and ophthalmological examination blood and urine sampling, ECG and X ray of heart lungs, and breasts and on subsamples, psychological psychiatric, dental somatometric and audiometric examination Furthermore, a dietary interview and a body composition examination were performed on subsamples This first presentation deals with the design and procedure of the study, analysis of observer variation and a comparison between responders (85%) and non responders (15%) The general design of the study, the sampling procedure and general methods of data collection were found to be adequate The responders were found to be representative for the population as a whole in most respects studied The results of this study to be reported in subsequent papers will in general allow conclusions regarding the population of 70 year-olds in Gothenburg

Although organized care of the old and debilitated and of the chronically ill has existed in most countries at least since the Middle Ages the increase of medical knowledge and technical resources during the 20th century has been mainly focused on acute diseases in children and in adults in so called productive ages while the basis for clinical work in the elderly population (geriatric medicine) is rather weak

The present knowledge of human ageing processes in general i e in gerontology is limited Basic facts which allow a distinction between symptoms and signs of ageing processes per se and those of diseases in elderly individuals are to a large extent lacking A marked absolute and relative increase of the number of elderly will occur in Sweden and in

The study was planned and organized by the authors and has been performed under their leadership (project leader A Svanborg) and with the participation also of E Andersson Ph D G Andersson secretary Å Brandberg M D Å Bruce M D E Deichgraber M D H Djurfeldt M B A A M Ek registered nurse R Jagenburg M D M Korsan Bengtsen M D I L Landin dietician M Lawenius B A T Lewin M D S Lindman B A M Munkby M D V Nilsson registered nurse G Persson M D K Soderberg registered nurse K Åstrand M D T Österberg D D S The *reference group comprised representatives of the* following institutions Clinical Chemistry Clinical Nutrition Hygiene Medicine II Ophthalmology Prosthetics Psychology Psychiatry (Sahlgren s Hospital) and Roentgenology III of the University of Gothenburg the Gothenburg Medical Services Administration and the Gothenburg Administration of Social Services

many other countries up to the 21st century This fact emphasizes the urgent need for better knowledge of ageing processes

The present living conditions for the elderly in the community and the need for subsidies aimed at decreasing the risk of physical inactivity and social and mental isolation should also be more thoroughly considered It is necessary to increase subsidies for preventive and prophylactic medical care also for the elderly in the community

A broader knowledge in geriatrics gerontology and sociology in older ages necessitates epidemiological studies Certain population studies in the elderly have in fact been performed in other countries (5 7 12) and in Sweden (8 10 14 15 27 28)

The present study which is cross sectional and will be continued prospectively is characterized by the sampling of a large group of relatively old people 70 years of age and by a design that will allow conclusions concerning ageing processes epidemiology of diseases and social background factors which might influence the development of ageing and disease

The main objectives of the study were 1) To make a survey of the social and medical conditions of the population 2) To obtain basic data for planning the care of the elderly 3) To contribute to the knowledge of normal ageing processes and of normal criteria within the age group 4) To offer the subjects a thorough medical examination

SOME DATA ON THE CITY OF GOTHENBURG

Gothenburg the second largest city in Sweden is situated on the West coast Characterized for many decades by its harbour and industries its most important industries have been and still are shipbuilding and motor industry Moreover Gothenburg plays an important role in the school and university life of Sweden The climate is coastal the Gulf Stream making it relatively mild in winter The mean temperature varies between about −4 and +18°C during the months of the year

The population in 1901 and 1902 in what was then Gothenburg totalled 132 111 and 133 625 respectively and amounted in 1971 and 1972 to 448 792 and 439 656 inhabitants respectively In 1901–02 the sex distribution of the inhabitants showed a surplus of women 60 783 men and 72 328 women (1901) and 61 533 men and 72 092 women (1902) This surplus has decreased so that the distribution was 221 432 men and 227 360 women in 1971 and 216 312 men and 223 344 women in 1972 The gainfully employed population in 1970 was about 195 000 distributed mainly between manufacturing 73 000 public services 54 000 trade 32 000 and communications 22 000 A very important role in manufacturing in Gothenburg is played by the shipyards which in 1971 delivered 17 ships with a total dead weight tonnage of 1 456 447 The two chief branches of manufacturing i e transportation equipment and metal-machine-electronics industry occupied 23 000 and 14 000 persons respectively (29)

PROCEDURE

Planning of the study took place during the period Jan 1969–Feb 1971 A pilot study was performed in Feb 1971 Further planning was completed in Sept 1971 when data collection started This period continued with short intermissions during Christmas 1971 Easter and July–August 1972 and was completed in Sept. 1972

Definitions

Some terms occurring frequently in the text are defined as follows *Propositus* a subject selected by the sampling procedure to participate in the study *Proband* a subject not only selected but also participating in the study Proband is the same as *responder* in contrast to a *non responder* who is a subject selected but not participating in the study Moreover a proband may be a complete proband i e participating in all intended parts of the study according to the sampling procedures or an incomplete proband *participating in one or more but not* in all the intended parts of the study

Staff and training of the personnel

The standardization of methods used by the physicians started during the planning period and was tested during the pilot study As all medical specialities were not represented two of us (S R and B S) were specially trained by other specialists in certain investigation techniques such as palpation of the prostatic and mammary glands and examination for inguinal and femoral hernia and ECG coding The specially employed personnel in the examination group working in the hospital (1 registered nurse 1 secretary and 1 auxillary) were trained during the pilot study and during a 2 week period immediately before the main study

Pilot study

During two weeks in Feb 1971 a pilot study was performed comprising 50 systematically selected 70-year-old propositi (42 actually participated) The aim of this pilot study was to test the feasibility of the study design of the individual questionnaires of the daily time schedule and other practical aspects and to give some preliminary training of the personnel especially regarding standardization of methods Only a minor revision of the study design was made for the main study No information from the pilot study has been included in the data bank of the main study

Sampling

A representative systematic sample of 70 year-old people in Gothenburg was obtained from the Revenue Office Register in the following way Those people were chosen who were registered for census purposes in Gothenburg

on the date of sampling and who were born during July 1st 1901–June 30th 1902 on dates ending with 2 5 or 8

In order to minimize non response due to deaths between date of sampling and date of investigation the sampling was performed in four steps on different dates (date of birth/date of sampling) July 1st–Sept 30th 1901/Aug 9th 1971 Oct 1st–Dec 31st 1901/Sept 28th 1971 Jan 1st–March 31st 1902/Dec 6th 1971 April 1st–June 30th 1902/March 10th 1972

The propositi were numbered consecutively (1, 2 3 4 5 1 2 etc) to make subsampling possible In the following those propositi who took part in the investigation are referred to as probands 1 2 3 4 and 5 respectively according to this listing

A representative systematic sampling of a control group of 70 year-old people was performed in Sept 1972 i e during the last month of data collection This sample comprised people born during July 1st 1901–June 30th 1902 on dates ending with 0 or 1 ($n=828$)

Offer to participate in the study

Letters were mailed to all propositi offering participation *in a health control particularly designed for elderly* people Sets of letters were mailed intermittently during the course of the whole study This first letter included a short description of the study design and the presumed advantages for the propositus to participate It was stressed that a nurse would get in touch with the propositus within a few days

Not more than a week after this letter had been mailed the nurse working with the home call part of the study tried to get in touch with the propositus by telephone or made a first visit to the given address Then a suitable time was agreed for the home call Precautions were taken so that the home call (or the later examinations) would not be arranged within a week before or after the birthday of the propositus

If the propositus refused to participate at this first contact the nurse tried to get in touch with him later on and several attempts were made to pursuade the propositus to participate and thus become a proband Moreover one further letter was mailed to propositi refusing to participate or who had not been possible to get in touch with in connection with the first letter The second letter resulted in participation of another 10 propositi (5 males and 5 females)

Home call

This part of the study was performed by two registered nurses who during the pilot study and a 2 week period before the main study had been trained together in asking the questions of the questionnaire and evaluating answers of different types The propositi who were to be interviewed by the two nurses were chosen in a systematic way from the list of propositi

Home calls by the nurses were performed Mondays to Fridays from 8 a m to 4 p m At the home call lasting about 1½ hours the nurse first completed or corrected data obtained at the sampling procedure regarding full name address marital status and other basic personal data Then an interview was performed based on a questionnaire consisting of 151 questions The questions concerned e g information and opinions about dwelling conditions economy social and physical communications previous migration educational level previous and any present professions need for care consumption of health care and drugs Observations and measurements were done of illumination at the habitual site of reading and a simple test of visual acuity was performed At the end of the interview the nurse estimated the collaborativeness of the proband and his level of orientation regarding own name date of birth and present day of week

At the end of the home call a date was agreed for the coming examination at the hospital and the proband was offered transport by taxi or ambulance if necessary Instructions were given regarding the test for bacteriuria (Cultube®) to be performed 24 hours before examination at the hospital and brought there and regarding collection of faeces Both written and oral information was given about the necessity of coming to the examination at the hospital without having eaten drunk or smoked later than 10 p m the preceding day One or two days before the examination at the hospital the secretary reminded the propositus of this agreement by telephone

Data on officially registered incomes for the year 1971 community rent allowances in 1971 and registrations at Gothenburg Temperance Board up to 1972 were not asked for from the proband but were obtained from official registers by special permission

Examinations at the Out patient Department of the Geriatric Hospital

The majority of the probands arranged transport to the hospital themselves For 52 probands we arranged transport by taxi *for a further 36 ambulance or special handicap* transportation was arranged and 29 were brought to the hospital by car by members of the staff

The examination took place at the Out patient Department of Vasa Hospital Some procedures i e general medical and ophthalmological examination blood and urine sampling ECG and X ray of heart lungs and breasts were performed on all probands others i e psychological psychiatric dental somatometric and audiometric examination on subsamples A secretariat was set up for catalogueing the data collected Part of the personnel also served as hostesses The probands had access to a special room where they had lunch and coffee

Procedures performed on all probands

In this first publication concerning the study the methods used will generally not be described in detail A more thorough description will be given in forthcoming reports on separate sections of the study

Urine and blood sampling and examinations Blood samples were drawn from an antecubital vein without stasis in the sitting position Blood was collected in one 5 ml EDTA glass tube used for determination of B Hb B-erythrocytes volume fraction (hematocrit) B-erythrocytes ESR and B leucocytes particle concentration (WBC) in five 10 ml heparine tubes which were immediately centrifuged and in two 10 ml glass tubes where the blood was left to clot for three hours at room temperature and then centrifuged Serum and plasma which was not

analyzed fresh were stored at −18°C in glass ampoules Capillary blood was collected from the finger tip for estimation of blood glucose level

Urine specimens were examined immediately after voiding with regard to the occurrence of albumin glucose blood ketone bodies and a rough estimation of urinary pH (Labstix®) The chemical laboratory determinations performed so far were B Hb B-erythrocytes—volume fraction (hematocrit) B leucocytes—particle concentration (WBC) B-erythrocytes—ESR B glucose B folate P folate P-cobalamines (vitamin B_{12}) P-bilirubin P alkaline phosphatases P aspartate—aminotransferase P alanine—aminotransferase P sodium P potassium S-calcium S phosphate P-magnesium S iron S total iron binding capacity (TIBC) S protein S-creatinine S urea P-cholesterol P total phospholipids P lecithin P lysolecithin P sphingomyelin P-cephalin P triglycerides P free fatty acids and S-urate

ECG Leads I II III aVR aVL aVF CR_1 CR_2 CR_4 CR_5 CR_7 were recorded after 10 min rest in the supine position The ECGs were coded according to the Minnesota code adapted to CR leads by the Scandinavian Committee on ECG Classification (25)

Mobility and nursing load estimation The mobility of the proband was graded according to the method of Gillner et al (11) and the nursing load according to Hultén et al (17)

Hearing ability examination Besides the more thorough audiological investigation performed on a subsample (see below) the ability to hear whispering and ordinary conversation was examined at a distance of 5 m or—if the proband did not hear at that distance—of 1 m

Ophthalmiatric examination In all probands visual acuity and refraction were determined Furthermore ophthalmoscopy and eye ground photography were performed after dilating the pupils

General medical examination From a practical point of view it was necessary with two investigators The questionnaire contained maximally 157 questions and subquestions A proband without present symptoms answered 69 questions

The somatic examination performed on all probands was standardized and comprised inspection of the body surface performed when the patients wore only short underpants percussion of lungs palpation of radial artery lymph nodules thyroid gland breasts abdomen and examination of the male probands for inguinal and femoral hernia Moreover the somatic physician performed auscultation of heart and lungs as well as rectal exploration

Arterial BP was measured after 5–10 min talking in the sitting position according to the recommendations by the WHO (32) A mercury manometer with a cuff 14×53 cm (rubber bag 12×30 cm) was used The diastolic BP was recorded at the point of muffling (phase 4) and at the point at which the sound disappeared (phase 5)

Roentgenological examinations Conventional roentgenological examination of heart and lungs and soft tissue roentgenological examination of the breasts in females according to Gros (13) were performed Heart volume was determined according to the method described by Jonsell (18)

Procedures performed on subsamples

Psychological examination Probands 1 and 2 were examined by the psychologists The examination of proband 1 mainly concentrated on the study of personality variables while proband 2 was examined as to intellectual capacity and capability

The following tests were performed individually To measure vocabulary ability the ability in drawing logical conclusions by the use of reasoning and figure classification the spatial ability and sensory motor speed and coordination the SRB 1 (6) SRB 2 (6 30) SRB 3 (6) and Ps if (6 30) were used To measure the intellectual capacity the logical test with Raven s matrices was used (24) and to measure the general personality of probands 1 a projective test (33) was used Furthermore a special inventory test with given alternative answers was performed

Psychiatric examination The subsample which was examined by a psychologist also had a psychiatric examination based on an interview comprising 108 questions After the interview an overall psychiatric characteristic of the proband was made Further studies were performed with a postal questionnaire as described below

Roentgenological examinations In probands 3 and 4 ortopantomography was performed and in a further subsample bone density of the radius was calculated according to Åstrand et al (34)

Odontological examination The dentist examined probands 3 and 4 Special interest was directed to the mucous membrane of the mouth On a further subsample there was performed a special stomatognatic investigation as well as an analysis of amount composition and rheology of saliva

Audiological examination Probands 4 and 5 were interviewed concerning a subjective comprehension of their hearing ability The examination also included conventional tone and speech audiometry The tests were performed in a sound proof test room by an audiometrist

Somatometric examination The somatometry part of the study included determination of 51 anthropometric measurements

Information to probands and physicians

At the end of the examination day all the probands were informed about the results then obtained A few weeks after the hospital examination the probands got information about the remaining results mostly by letter but in some cases by telephone On the same occasion a letter with relevant results of the investigation was sent to the patient s own doctor if any

Some probands with signs and symptoms of disease were controlled by members of the research staff at the Geriatric Out patient Department of the hospital Others were admitted to other specialists However most of the patients were further controlled by their own doctors

Subsequent examinations

Dietary intake examinations In probands 3 and 4 diet interviews (24 hour recall and dietary history) were performed by the dietician in the proband s own home in 65% within two weeks and in 74% within four weeks after the hospital examination The calculations were

Table I *Interobserver variation regarding blood pressure measured in 17 probands by two physicians*

	Systolic	Diastolic 4	Diastolic 5
B S			
10 mmHg higher value	1	1	1
5 mmHg higher value	5	4	3
Equal	6	9	10
5 mmHg lower value	2	2	3
10 mmHg lower value	3	1	0

done with a desk calculator according to a system worked out at the Department of Clinical Nutrition University of Gothenburg In these probands the 24-hour urinary excretion of nitrogen creatinine sodium potassium and calcium was analyzed

Body composition examination In a further subsample (n=105) of these probands total body water was determined with an isotope dilution method with tritiated water as described from the Department of Clinical Nutrition University of Gothenburg (20) *Total body potassium* was determined with a whole body counter using K^{40} (26) Body cell mass intracellular water extracellular water and body fat were then calculated

Psychiatric postal questionnaire At the end of the psychiatric examination three personality inventories Maudsley personality inventory MPI (9) Marke Nyman test MNT (23) and Cesarec–Marke personality schedule CMPS (4) with altogether 305 questions were given to the proband to be answered at home and mailed back

Data handling and processing

After the completion of the data collection data were handled as follows checking of completed forms checking of identification number and number of forms checking of transcription from forms into code rings punching control punching listing of punch-cards checking of listings repunching of incorrect punch-cards computer programming transcription of data into magnetic tape storage processing of rough frequencies and output routine rough frequency classifying and further processing

In the following presentation of observer variation and response only those differences are regarded as significant which have a significance level of $p<0\ 01$ However in statements of non significance information is given whether the level is $p>0\ 01$ or $p>0\ 05$

INTRAOBSERVER VARIATION

Determination of heart volume

The determinations of heart volume were performed by the same roentgenologist during the whole study Intraobserver variation was studied in 100 consecutive cases read on two different occasions—four weeks apart—by the roentgenologist The difference between the two readings was $-0\ 3\pm2\ 62$ ml (mean ±S E) This difference was not significant ($p>0\ 05$ Student s t test)

Classification of ECG

Intraobserver variation was studied in S R and B S who recoded 50 and 60 consecutive ECGs respectively 3 weeks after the proper codings had been finished In all but one of these 110 cases the two codings were identical In one case in one physician a borderline ST depression was judged to be more than or equal to 0 5 mm (code 4 4) at the recoding instead of less than 0 5 mm (code 4 5) This variation was judged to be of minimal importance

Calculation of intake of energy and nutrients

The same dietician who interviewed the probands and originally calculated the intakes of energy protein fat carbohydrates potassium calcium iron vitamin A thiamine riboflavin and ascorbic acid recalculated the 24 hour recall and the diet history values of intakes of energy and nutrients in 40 consecutive probands No significant differences between the two values were observed ($p>0\ 05$ Student s t test)

INTEROBSERVER VARIATION

Social interview

The proportions of 18 alternative answers were tested in each sex regarding differences between the two nurses Significant differences ($p<0\ 01$ χ^2 test) were obtained regarding the answers to questions regarding the following items Standard of dwelling unit (men) Do not dare to go out in the evening (men) Social contact (women) Difficulties in getting in touch with a doctor (women) Noisy surroundings (men) In-door temperature in winter (women) There was no significant difference ($p>0\ 05$) in the more objective testing of the visual acuity by means of Jaeger (Student s t test)

Blood pressure measurement

One of us (B S) examined probands 3 4 and 5 and one of us (S R) probands 1 and 2 Interobserver variation was studied in the following ways (Tables I and II)

1) Seventeen consecutive probands 2 and 5 were examined by both physicians during June 1972 Table I shows that no bias was observed The mean values for BP were 161 2 (systolic) 97 1 (diastolic 4) and 93 8 mmHg (diastolic 5) for B S and 161 5 96 5 and 93 2 mmHg respectively for S R The differences between the two sets of readings were $-0\ 3\pm1\ 45$ (mean±S E) $0\ 6\pm1\ 13$ and $0\ 6\pm0\ 95$ mmHg respectively ($p>0\ 05$ Student s t test)

2) Table II shows BP values for all probands 1 and 2 (examined by S R) and all probands 3 4 and 5 (examined by B S) There were no significant differences between these two groups according to their BP readings ($p>0\ 05$ Student s t test)

This comparison of BP values in the two groups of probands gives of course only an indirect view of interobserver variation Identical values might be the result of for example higher real BP in one group and biased

Table II *Blood pressure in probands 1 and 2 and probands 3 4 5, respectively*

	Males				Females			
	Probands 1 2 (*n*=168)		Probands 3 4 5 (*n*=281)		Probands 1 2 (*n*=224)		Probands 3 4 5 (*n*=298)	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Systolic	160	26 4	160	24 0	172	24 0	169	23 4
Diastolic 4	95	13 8	94	11 6	98	13.5	97	11 2
Diastolic 5	92	14 2	91	12 2	95	13 7	93	12 1

readings by the two observers working in the opposite direction However in view of the relatively large groups such an explanation is not probable

Classification of ECG

ECG codings were performed by S R in probands 1 and 2 and in probands 3 4 and 5 by B S Interobserver variation was studied in the following ways

1) B S and S R coded independently ECGs from 50 consecutive probands In two cases the readings were not identical In one case one of us did not notice a borderline low T wave in lead II (1 0 0 was coded instead of 5 4) In the other case the other one of us misjudged a borderline ST depression as being more than or equal to 0 5 mm (code 4 4) instead of less than 0 5 mm (code 4 5) These false codings were judged to be of minimal importance

2) The number of probands 1 and 2 with Q and QS patterns types 1 or 2 ST depression types 1 2 or 3 atrial fibrillation or ventricular premature beats was compared to the corresponding number of probands 3 4 and 5 (Table III) There were no significant differences between these two groups the ECGs of whom were coded by the two investigators ($p>0\ 05$ χ^2 test)

The same judgement principles described for interobserver variation of BP measurement are applicable to this comparison too

RESPONSE

Altogether 451 males (86 6%) and 522 females (83 3%) had a complete examination giving an overall participation rate of 84 8% Another 19 males (3 6%) and 15 females (2 4%) participated in the home call part of the study altogether 3 0% The non participation rates concerning all parts of the study were therefore 9 8% (males) 14 4% (females) and 12 3% (total material) (Table IV)

Table V summarizes the reasons for non participation Ten males and 3 females died between the date of sampling and the date of investigation The refusal rate was 7 9% in males and 13 9% in females The reasons for no-participation in the hospital examination part of the study in those propositi who had a home call were in 3 males serious illness and in 16 males and in 15 females refusal for other reasons

COMPARISON OF PARTICIPANTS IN THE COMPLETE INVESTIGATION WITH NON PARTICIPANTS

Tables VI and VII summarize some data from the 973 participants in the hospital part of the study (451 males 522 females) compared to similar data from the 175 non participants (70 males 105 females) No significant differences were found between responders and non responders as to sex marital status proportion of propositi with registrations in the Temperance Board Register community rent allowances and income

In males 31 4% of the non responders and 42 4% of the responders had been subjected to in patient somatic care during 1966–72 This difference was however not significant In females there was the same tendency towards more hospital care during 1966–72 in responders (35 2%) than in non

Table III *Codings according to the Minnesota code of ECGs of probands 1 and 2 and 3 4 and 5 respectively*

	Males		Females	
	Probands 1 2 (*n*=168)	Probands 3 4 5 (*n*=271)	Probands 1 2 (*n*=223)	Probands 3 4 5 (*n*=291)
Q 1–2	6	22	7	9
ST 1–3	42	76	75	94
Atrial fibrillation	6	12	3	7
Ventricular premature beats	11	15	7	18

Table IV *Response*

	Males		Females		Total	
	(n)	(%)	(n)	(%)	(n)	(%)
Complete examination	451	86 6	522	83 3	973	84 8
Home call only	19	3 6	15	2 4	34	3 0
Non response	51	9 8	90	14 4	141	12 3
Total	521		627		1 148	

responders (21 9%) This difference was significant (Table VIII)

Table VIII also shows the occurrence of care at in patient and out patient psychiatric departments from the 1920s onwards in Gothenburg in responders and non responders among propositi 1 and 2 i e those probands who were examined by the psychiatrist and the psychologists There was no significant difference between the two groups in this respect

Table VI *Sex marital status and male propositi registered with Temperance Board among responders and non responders (χ^2 tests)*

	Responders		Non responders	
	(n)	(%)	(n)	(%)
Males	451	46 4	70	40 0
Females	522	53 6	105	60 0
	$p>0\ 05$			
Unmarried divorced and widowers (♂)	104	23 1	17	24 3
Married (♂)	345	76 5	53	75 7
	(2 data missing)			
	$p>0\ 05$			
Unmarried divorced and widows (♀)	291	55 7	54	51 4
Married (♀)	230	44 1	49	46 7
	(1 data missing)		(2 data missing)	
	$p>0\ 05$			
Registered with Temperance Board(♂)	77	17 1	15	21 4
Not registered (♂)	374	82 9	55	78 6
	$p>0\ 05$			

DISCUSSION

The non response rate is usually higher in population studies of older than of middle aged people (3 15 22) which limits the possibilities of generalizing from sample to population in higher age groups In the present study the non response rate was rather low Our opinion is that the home call was of fundamental importance for the good outcome of the present study with respect to participation The telephone calls to the propositi a couple of days before the investigation at the hospital seemed to further stimulate the propositi to participate

Differences between responders and non responders are common in population studies and the responders are thus at least in some respects not representative for the population Tibblin (31) for example showed that in a study of 50 year old males in Gothenburg the non participant group differed in income and marital status and was also more frequently registered in the Temperance Board Register Bengtsson et al (1) in a study of women in Gothenburg found that single women were to some extent overrepresented among the refusers In the present study however no significant differences were found concerning sex marital status income community rent allowances or proportion of probands registered in the Temperance Board Register Because earlier studies have indicated that people with certain socio economic characteristics might be overrepresented in the non response group (31) we found it interesting—in spite of the lack of significant differences in this respect on the 1% level between responders and non responders—to investigate the results more closely regarding possible trends A grouping of males who were not married belonged to the lower quartile group regarding income and were registered with the Gothenburg Temperance Board showed however no significant difference between the non response and response group ($p>0\ 05$ χ^2 test)

We considered it furthermore important to compare the consumption of hospital care for a

Table V *Causes of non participation*

	Males	Females	Total
Dead after date of sampling	10	3	13
Living far from Gothenburg	3	1	4
Inaccessible	2	2	4
Refusal	36	84	120
Total	51	90	141

Table VII *Income and community rent allowances (Sw cr) in responders and non responders (Student s t test)*

All 70-year-old Swedish citizens in 1971 had an old age pension of 5 314 (married) and 6 687 (single) Sw cr a year per capita Analysis of income showed that the old age pension was the main income for females Males often had income from other sources as well

	Responders		Non responders	
	Mean	S D	Mean	S D
Income				
Males	20 298	18 215	15 551	19 448
		0 05>p>0 01		
Females	*10 562*	*10 286*	*10 149*	9 238
		p>0 05		
Community rent allowances				
Males	705	1 041	928	1 083
		p>0 05		
Females	1 239	1 508	1 084	1 334
		p>0 05		

certain period in responders and non responders The higher proportion of somatic hospital care in the female response group might be due either to the fact that the non responders were healthier than the responders or that the female responders showed a more active interest in medical care and health control than those who refused to participate in our study

In the further analysis of the results of this study we will consider the possibility that the probands in other respects than those discussed in this paper were not representative for the 70 year-old population in 1971–72 It is possibly so in any population study with a non response that the non responders in some respect or other are different from the examined part of the population

In younger age groups a main purpose of medical population studies is besides so-called health control to reveal risk factors that might associate to future special diseases and if possible to eliminate or compensate for such risk factors At higher ages such risk factors can be expected to have already eliminated many individuals from the population or to have caused manifest symptoms of disease Search for and primary prevention of such more specific risk factors are therefore often less meaningful in higher ages On the other hand there may be reasons to believe that risk factors of a more non specific character such as physical inactivity and social isolation are more common in higher age groups

A main goal of the present study was to collect not only medical and psychological data but also a comprehensive body of information about previous and present social conditions of the *propositi* Thus comparatively much time and resources were spent on the home call The time spent on the home call about 1½ hours seemed to be sufficient to come into good contact with the proband and to collect the desired information but apparently not too long or tiring Collection of information on social conditions through an interview at a home call instead of asking questions at a hospital visit or by means of a postal questionnaire has the important advantage that the validity of much of the information can be *checked up by the interviewer* e g *concerning data* on standard of the dwelling distance to bus stop etc Moreover the proband s level of orientation may be better estimated at a home call with the proband in his habitual situation

However interviewing about certain socio-economic items is not necessary as Sweden has a

Table VIII *Somatic in patient care during the period 1966-01-01 to date of sampling and psychiatric in and out patient care in responders and non responders* (χ^2 *test*)

	Responders (n)	(%)	Non-responders (n)	(%)
Males				
Somatic in patient care	191	42 4	22	31 4
No somatic in patient care	260	57 6	48	68 6
		p>0 05		
Females				
Somatic in patient care	184	35 2	23	21 9
No somatic in patient care	338	64 8	82	78 1
		p<0 01		
Male probands 1 and 2				
Psychiatric care	29	17 3	5	19 2
No psychiatric care	139	82 7	21	80 8
		p>0 05		
Female probands 1 and 2				
Psychiatric care	35	15 6	7	17 5
No psychiatric care	189	84 4	33	82 5
		p>0 05		

registration of e.g. income and community rent allowances and of contacts with the Temperance Board. Moreover, interviewing around such matters at the home call might result in non-participation in other parts of the study. The Temperance Board Register includes not only alcoholics but also occasional drinkers, e.g. because of drunken driving. This register has, however, been considered to give more valid information concerning abuse of alcohol than an interview (16). In the present study the diet interview performed after the hospital examination comprised a couple of questions concerning beer, wine and liquor consumption. The study thus comprises two methods aimed at a rough estimation of alcohol consumption and abuse of alcohol apart from the information obtained at the home call.

A small number of proposíti—mainly women—reacted against the words "Health control of the 70-year-olds in Gothenburg" printed on the envelope of the invitation letter. However, this was in no case the cause of refusal, but we will in future studies avoid such chronological labelling.

In a study of elderly individuals, transportation of patients with a physical handicap must be arranged. Furthermore, arrangements must be made to escort some of the proposíti to the hospital, namely those who feel anxious and uneasy about leaving for a whole-day excursion by themselves.

One problem in studies like the present one is to make sure that the probands are fasting when coming to the hospital examination. It is difficult to definitely prove that this is the case. In order to motivate the probands to be fasting they were explicitly informed that breakfast would be served in the hospital immediately after the blood sampling. Furthermore, at the telephone call a couple of days before the hospital examination, the necessity of being fasting was again emphasized.

The programme for the hospital part of the study was changed after observations made in the pilot study, so that fewer pauses were included during the day. It was then experienced as neither tiring nor tedious. In the pilot study it was found necessary to have regular, weekly staff meetings with careful minutes, where unexpected interview answers, etc. could be discussed for proper coding.

Diurnal and seasonal variations might be present concerning some of the variables studied. In order to minimize the importance of diurnal variation, the different parts of the examination were held during approximately the same time of day throughout the present study. The influence of seasonal variation is impossible to avoid in studies like the present one. However, by spreading the study throughout all seasons of the year, the consequences of seasonal variation may be compensated for when studying groups of probands.

The importance of interobserver variation has been repeatedly stressed by many authors, e.g. Lowe and McKeown (?1), Keys et al. (19) and Tibblin (31) for measuring of blood pressure and by Blackburn (?) for interpreting ECGs. For practical reasons it was necessary to have two observers in some parts of the present study, i.e. in the social investigation made by two registered nurses and in the general medical examination and in the coding of ECGs. The present study has revealed some interobserver variation in the social part of the interview between the two registered nurses regarding some questions, the answers to which and the evaluation of the answers might have been influenced by the personality of the interviewers. These differences could not be explained by social differences between the two groups interviewed by the two nurses, as judged from income and geographical distribution within the city, which were found to be similar in the two groups. The observed interobserver variation diminishes the possibilities of drawing conclusions from that part of the interview with such "subjective" answers. Most of the questions were, however, constructed in such a way that the answers could be given and evaluated "objectively" and only about every 4th-5th answer might have been given or evaluated "subjectively". Registrations performed at the home call, e.g. visual acuity, were similar in the two interview groups. Regarding coding of ECGs according to the Minnesota code, other studies have shown great interobserver variation (?). It is our opinion that the low interobserver variation in the present study was due to thorough training before the study.

In studies of short duration, the problem of intraobserver variation is commonly less serious. During studies of longer duration the possibilities of greater intraobserver variation must be taken into consideration. In the present investigation it was possible to study the intraobserver variation only in a very limited number of observations and calculations. The reason why the intraobserver variation studied was found to be so small might also be the systematic training before the start of the study.

In a population study including medical examinations many of these might be performed with high accuracy by specially trained personnel other than physicians In a study like the present one however the physicians who are responsible for the final message to the patient, have to be very much involved in the examinations in order to get to know the probands and to be able to give the information in an adequate and individually adjusted way The experience of the present study indicates that such a need of personal contact in the role of the physician with the proband is of great importance Very few medical examination procedures could therefore be handed over to para medical personnel

Our conclusion from the present treatment of the material is that the general design of the study the sampling procedure and general methods of data collection were adequate Furthermore as far as we can see now the results of this study will in general allow conclusions regarding the population of 70 year olds in Gothenburg The reason for this is that the responders seemed to be representative for the population as a whole in most respects studied

Results from the population study as such and from the health control part of the study will be presented later

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Artificial Hyperventilation during 21 Years in Three Cases of Complete Respiratory Paralysis

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ABSTRACT Three patients with paralytic poliomyelitis have been ventilated via tracheostomy with uncuffed silver cannula for 21 years, with high tidal volumes of atmospheric air (8 3, 7 2, and 5 4 ml/kg b wt), at a frequency of 20, passive expiration, and without periodic hyperinflation No pulmonary complications were seen during the whole of this period The total compliance was significantly decreased The pulmonary physiological shunt relative to the total pulmonary blood flow (Q_s/Q_t) was slightly increased PaO_2 was nevertheless normal probably due to a high alveolar PO_2 caused by the hyperventilation The physiological dead space relative to the tidal volume (V_D/V_T) was within the normal range but V_D was high in one case Two of the patients disclosed an extremely low CO_2 production and a $PaCO_2$ averaging 12 mmHg, with small fluctuations during a 24-hour study This profound respiratory alkalosis was only partly compensated in the arterial blood (pH 7 54 and 7 50), suggesting a new state of acid–base equilibrium The cerebrospinal fluid lactate was significantly increased to about 4 mmol/l but the patients revealed no signs of impaired cerebral function A reduction of the degree of hypocapnia by the use of a mechanical dead space is recommended

During the epidemic of poliomyelitis in Copenhagen in 1952 264 patients were submitted to tracheostomy and 232 of them were treated with positive pressure ventilation (7) Four years later 24 of 138 survivors were considered to be chronic respirator patients Nine of these chronic patients died during the following 17 years Among the 24 chronic patients five developed diabetes and more than half urinary calculi Thirteen of the 15 chronic respirator patients who are still alive require ventilation constantly

In the acute stage normoventilation was aimed at Afterwards the use of uncuffed silver cannulas i e uncontrolled leakage demanded a high minute ventilation which in addition made the patients more comfortable

It has been demonstrated that prolonged respirator treatment requires a substantial increase of both ventilation and inspiratory PO_2 (14) As a matter of fact the above mentioned paralysed patients have been hyperventilated with atmospheric air without revealing any clinical signs of hypoxia or pulmonary complications Thus a study of the pulmonary function and the effects of and adaptation to the induced and sustained hypocapnia seemed justified

PATIENTS AND METHODS

Informed consent to the present study was obtained from three patients

Case 1

A male patient was admitted to the hospital at the age of 32 with poliomyelitis causing total respiratory paralysis and complete tetraplegia He regained slight function of four fingers on the right hand normal deglutition and facial activity The rest of his muscles remained paralysed He has been continuously ventilated through a tracheostomy (silver cannula) since the acute phase of his disease and has been free from pulmonary complications during the whole period Toilette of the airways is usually performed twice daily Diabetes mellitus was diagnosed seven years ago first treated with insulin and thereafter with tolbutamide

Case 2

When five years old the patient was admitted to the hospital with polio She has residual total respiratory paralysis total paralysis of the upper extremities and except for the toes severe paresis of the l

Table I *Data of three patients with complete respiratory paralysis after poliomyelitis 21 years previously (Copenhagen epidemic 1952)*

Case no	Sex	Age (y)	Height (cm)	Weight[a] (kg)	Average of 24 hour study: BP (mmHg)	Pulse rate	Temperature (°C)	Hb (g/100 ml): Initial	After 24 h[b]	Decrease (%)	Serum creatinine[c] (mmol/l)
1	♂	53	176	69	150/–	68	36 2	14 0	12 6	10	3 3
2	♀	26	156	63	115/90	96	37 4	8 7[d]	6 8	22	8 0[d]
3	♀	39	173	68	115/70	76	36 8	12 9	10 6	18	2 5

[a] Pronounced reduction of the muscular component
[b] An average of 70 ml blood was sampled from each patient during the 24 hour study
[c] Limits of normal values 7 8–12 2
[d] Pyelolithotomy 16 days previously

She has been ventilated through a tracheostomy (silver cannula) ever since without pulmonary complications Tracheal toilette has been performed four times daily She has suffered from recurrent urinary infections recently complicated by renal calculi on both sides A pyelolithotomy was performed 16 days prior to the present study

Case 3

First admitted as a girl of 18 years suffering from poliomyelitis with persistent subtotal respiratory paralysis and nearly complete paralysis of one upper and both lower extremities She had a tracheostomy during the first year but then managed without for four years Since 1957

Table II *Ventilation and pulmonary function in three patients with complete respiratory paralysis*

	Case 1	Case 2	Case 3
Ventilation			
Total inflation volume leakage included (ml (BTPS))	900	825	725
Respiratory tidal volume (ml (BTPS))	575	455	365
Leakage/total inflation volume (%)	36	45	50
Respiratory tidal volume (ml/kg b wt)[a]	8 3	7 2	5 4
P_ECO_2 (mmHg)	5 0	7 0	13 5
CO_2 output (ml/min (STPD))	70	76	118
$PaCO_2$ during dead space determination (mmHg)	11	11	21
Frequency (inspir /expir =2/3) (per min)	20	20	20
Peak inspiratory pressure (cm water)	34	42	28
End inspiratory pressure plateau (cm water)	14	18	17
Arterial blood			
$PaCO_2$ during 24 h (mmHg)			
Mean	12	12	24
Range	10–15	11–16	18–30
pH during 24 h	7 50	7 54	7 39
Standard bicarbonate during 24 h (mmol/l)	16 2	16 9	17 6
P_{50} calculated from a venous sample (mmHg)[b]			23 5
2 3 DPG (moles/mole Hb tetramer)[c]		1 442	0 931
PaO_2 during ventilation with air (mmHg)	114	105	103
PaO_2 during ventilation with pure O_2 (mmHg)	450	389	433
Pulmonary function			
Total compliance (ml/cm water)[d]	41	25	21
V_D/V_T (%)	55	36	36
$\dot{Q}_s/\dot{Q}_t$ (%)[f]	13	16	14

Corrected for compression of gas within the ventilator and the connections to the patient s lower airway (3)
[b] PO_2 at which SO_2 is 50% normal value 27 1 (S D 1 05) in females
[c] 2 3 diphosphoglycerate normal value 0 83 (S D 0 10) in females (11)
[d] Respiratory tidal volume/end inspiratory pressure
[e] V_D=physiological dead space V_T=tidal volume $V_D/V_T=(PaCO_2-P_ECO_2)\times 100/PaCO_2$
[f] $\dot{Q}_s$=pulmonary physiological shunt (calculated venous admixture) $\dot{Q}_t$=total pulmonary blood flow $\dot{Q}_s/\dot{Q}_t$ estimated from P_AO_2 (=$P_IO_2-PaCO_2$) and PaO_2 during ventilation with 100% O_2 (10)

Table III *Cerebrospinal fluid (CSF) and response to $PaCO_2$ increase in three patients with complete respiratory paralysis*

Case no	CSF							Response to gradual $PaCO_2$ increase with unchanged ventilation			
	$PaCO_2$ (mmHg)	PCO_2 (mmHg)	pH	HCO_3^- (mmol/l)	Lactate (mmol/l)	Pyruvate (mmol/l)	Lactate/pyruvate	Initial	Sudden discomfort[a]	Period of increase (min)	Calculated CSF pH decrease[b]
I	11	19	7 35	10 2	4 16	0 238	17.5	14	22	17	0 10
2	12			–	3 88	0 310	12 5	11	24	27	
3								26	38	20	
13 normal adults											
Mean	40 3		7 32	23 6	1 54	0 124	12 3				
S D	1 1			0 9	0 31	0 010	1 9				

[a] All complained of progressive tiredness and dizziness until a sudden onset of extreme discomfort was provoked with severe headache and a feeling of suffocation

[b] According to the Henderson Hasselbalch equation assuming 2/3 of complete CSF PCO_2 equilibration (2)

she has been tracheostomized with a silver cannula Except for some hours daily during the first five years she has been ventilated either by cuirass or via tracheostomy throughout the whole 21 year period Renal calculi developed on both sides which ultimately led to nephrectomy on one side and pyelolithotomy on the other Two years ago hyperglycemia and glucosuria were found and were temporarily treated first with insulin and then with tolbutamide

During the last 12 years the three patients have been ventilated by a portable respirator (battery-operated weight 15 kg) volume-cycled working with fixed frequencies (18 20 and 22/min) and a modified sine wave pressure pattern The expiration is passive through a low resistance outlet and no periodic hyperinflation is employed The tidal volumes (V_T) have been essentially unchanged during the past years The leakage around the uncuffed silver cannula permits the patients to speak almost naturally

The present study covered a 24-hour period in each of the three patients The ventilation was kept unchanged except for a short period during which a cuffed tube was substituted for the usual silver cannula in order to evaluate the leakage the total compliance and the physiological dead space (V_D) V_T was reduced until the original $PaCO_2$ had been regained whereupon pressures and volumes were measured and expired air was collected and mixed for P_ECO_2 determination The pulmonary physiological shunt was estimated from PaO_2 measurements after the patient had been ventilated with pure oxygen for 20 min The cerebrospinal fluid (CSF) was obtained by lumbar puncture during steady state conditions The reaction to a gradual rise of $PaCO_2$ was observed during an unchanged ventilation with a stepwise increase of inspired CO_2 concentration

A teflon-covered glass electrode (Radiometer Denmark) was used for the PCO_2 measurements The $PaCO_2$ was determined 13 times during the 24-hour period The pH was measured with an Astrup glass electrode (Radiometer Denmark) whereas a Clark electrode (Radiometer Denmark) was used for the PaO_2 measurements The precautions stated by Leusen (8) were taken into account for the CSF measurements Enzymatic methods were used for determination of 2,3-diphosphoglycerate (2 3-DPG) (11) and for lactate and pyruvate concentration (1)

Normal values for CSF (lumbar) acid–base variables were obtained in 13 normal adults suspected of herniation of a lumbar disc

RESULTS

Some characteristics of the patients studied are summarized in Table I ECG and lung parenchyma in the X rays were normal in all patients The characteristics of the ventilation employed and the associated changes in arterial blood are summarized in Table II which includes calculations concerning the pulmonary function The $PaCO_2$ values measured during sleep did not differ significantly from values measured while awake The CSF changes associated with prolonged hypocapnia are compared with normal CSF (normocapnic) values in Table III which also shows the maximal $PaCO_2$ increase tolerated by the patients

DISCUSSION

Following the long lasting paralysis a marked reduction of the body s muscular component is present in these patients Consequently the circulating blood volume is reduced This is substantiated by the pronounced fall in Hb concentration associated with the blood loss of about 70 ml (Table I) Another consequence of the reduced muscular

mass is the low level of serum creatinine normally found in these patients (Table I)

As shown in Table II a pronounced hypocapnia was revealed The variations in $PaCO_2$ during the 24-hour study were limited Thus the leakage of air through mouth and nose which comprises from one third to one half of the volume delivered from the respirator is unchanged during sleep In normal man a V_T of 10 ml/kg b wt given with a frequency of 20 would cause a $PaCO_2$ reduction to the mid twenties The lower $PaCO_2$ values actually found are due to a low CO_2 production in the present paralysed patients (Table II)

The pronounced respiratory alkalosis (cases 1 and 2) is only partly compensated by a decreased standard bicarbonate (Table II) To obtain complete compensation standard bicarbonate would have to be decreased to 13 2 and 12 3 mmol/l respectively The demonstration of an unchanged renal maximal reabsorption of bicarbonate during prolonged hyperventilation in man (high altitude) (9), supports the view that a new state of acid-base equilibrium has been obtained in cases 1 and 2 The more moderate hypocapnia in case 3 was completely compensated which is in agreement with other studies (16)

Hypocapnia will cause an immediate left ward shift of the oxyhaemoglobin dissociation curve (Bohr effect) which after some hours will be followed by a right ward shift (i e normalization) associated with an increased intraerythrocytic concentration of 2 3 DPG (6) In case 3 a slight left ward shift (lowered P_{50}) and a normal 2 3 DPG level were found (Table II) Thus no compensation to the Bohr shift mediated by a 2 3 DPG increase was detectable The raised 2 3 DPG level in case 2 might be due to both respiratory alkalosis and anaemia (Tables I and II) (6)

The total compliance (lungs and chest wall) in anaesthetized and paralysed man in the supine position has been reported to average 72 ml/cm water (range 62–90) (10) Thus a significant decrease in the total compliance was found in the present study (Table II) This decrease could be related to the lowered functional residual capacity found in identical patients (4) or to stiffness of the chest wall

V_D/V_T (%) is normally roughly estimated as 33+age/3 (10) The present V_D/V_T values are thus normal but the V_D actually found in case 1 was high (Table II) The pulmonary physiological shunt (calculated venous admixture) relative to the total pulmonary blood flow (Q_s/Q_t) has been estimated from a calculated alveolar PO_2 and an observed PaO_2 during ventilation with pure oxygen (Table II) (10) The present estimates are based on normal values for the oxygen content of mixed venous blood This assumption has been substantiated in identical patients (4) In normal man breathing pure oxygen Q_s/Q_t has been found to average 9 3 % (S D 1 7) (19) Thus the present patients all have a slight increase in right to left shunting (Table II) The finding of completely normal PaO_2 values during ventilation with air in all three patients despite an increased shunting is explained by an increase in the alveolar PO_2 due to hyperventilation

A nearly complete CSF pH adaptation (normalization) was found in case 1 (Table III) This was brought about by a decreased CSF bicarbonate concentration associated with an increase in lactate concentration A decreased CSF bicarbonate means a decreased buffering capacity against CO_2 (5) The lactate accumulation in CSF during profound hypocapnia is postulated to be at least partly a sign of brain hypoxia associated with pronounced vasoconstriction (13) In the present study there were no clinical signs indicating brain hypoxia Thus the increased lactate formation in neuronal tissue might be explained as a homeostatic response tending to normalize the CSF pH The normal PCO_2 gradient from CSF to arterial blood (8 mmHg) found in case 1 suggests a normalization of the cerebral blood flow during sustained hypocapnia (15)

The sudden extreme distress provoked by a gradual $PaCO_2$ increase of 8, 13 and 12 mmHg respectively (Table III) might be related to the concomitant decrease in CSF pH Acidosis in CSF causes a sudden cerebral vasodilatation (15) giving rise to headache whereas the feeling of suffocation might be related to a stimulation of the central chemoreceptors In normal man a CSF pH decrease of 0 02 U will cause a significant hyperventilation (18) Assuming the presence of two-thirds of the complete PCO_2 equilibration in CSF associated with the induced $PaCO_2$ increase (2) a CSF pH decrease of 0 10 is calculated in case 1 A decreased sensitivity of the central chemoreceptors might therefore be suggested In another study of patients with ventilated paralytic poliomyelitis (12) the responses of the sensation of shortness of breath to reduced tidal volumes and the reflex hyperventilation from the inhalation of CO_2 corresponded to those of normal man

CLINICAL COMMENT

During artificial ventilation $\dot{V}_D$ and the $\dot{Q}$ increase (14) The present study has however shown that V_D/V_T remains normal during hyperventilation even when this has lasted for several years Furthermore oxygenation of arterial blood is apparently not impaired and pulmonary complications are avoided probably because the patients are ventilated with high volumes of air

Drawbacks could be related to the associated profound hypocapnia Except for CSF the patient can not compensate completely for a profound respiratory alkalosis The high incidence of renal calculi might be related to immobilization and to high urinary pH associated with periodical falls in $PaCO_2$ Because of the high incidence of diabetes mellitus a causal relationship to sustained hypocapnia might be suspected

Thus during prolonged respirator treatment high V_Ts have to be used The significance of the high respiratory frequency actually employed has not been settled A restriction of the induced hypocapnia is however recommended by the addition of a mechanical dead space The appropriate size of this dead space can be read off from a nomogram (17) bearing in mind that the CO_2 production may be decreased

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Intestinal Decarboxylation of Orally Administered L-dopa

Influence of Pharmacological Preparation Dose Magnitude Dose Sequence and Food Intake

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ABSTRACT The intestinal decarboxylation and the absorption of orally administered L-dopa have been studied in 20 parkinsonian patients in different conditions The decarboxylation generally amounted to 50–70% of the dose given i e only 30–50% of orally administered L-dopa reached the general circulation The shape of the plasma concentration curve varied individually and with the resolvation time of the type of pharmacological preparation given No obvious difference in the degree of intestinal decarboxylation was observed when tablets with different resolvation times were given but the net absorption of L-dopa was somewhat greater when it was given as a solution The decarboxylation in the intestinal organs was found to be rather constant at different times of the day Isocaloric meals, whether rich in protein or carbohydrate, caused a delay in absorption of L-dopa, but did not change the degree of decarboxylation Diurnal variations in the clinical response to L-dopa could, thus not be related to diurnal variations in the decarboxylation in the intestinal organs The delay in L-dopa absorption after meals may be misinterpreted as lack of response At dosage with short intervals, the delay may cause an additive effect of two doses

The clinical response to a certain dose of L-dopa varies markedly not only between individuals but also in one and the same individual during the day Earlier studies showed that the marked interindividual differences in dose requirement apparently could not be related to any difference in the degree of decarboxylation in the intestinal organs (7) Lander (9) suggested that there were differences in clinical response which would be attributed to the pharmaceutical preparation used

The aim of the present investigation was to study whether the degree of intestinal decarboxylation of L-dopa is influenced by the magnitude of the dose by differences in the resolvation time of the pharmacological preparation and by food intake Furthermore we wanted to study whether the rather frequently observed diurnal variation in the L-dopa effect could be correlated to differences in the intestinal decarboxylation

MATERIAL

Twenty parkinsonian patients were investigated 8 women and 12 men Their mean age was 65 years (range 48–78) and their mean body weight 69 kg (range 49–94) Three of the patients (nos 1–3) had not been treated previously with L-dopa patients 4–19 had been treated with L-dopa for 12–66 months and patient 20 for two months with maintenance doses of 1 4–6 0 g The patients appeared to have normal liver and renal functions as judged from anamnestic data routine laboratory tests and from clinical findings One patient (no 9) had undergone gastric resection ad modum Billroth II 22 years earlier He was studied in order to determine the possible effect of a subtotal gastrectomy on the L-dopa absorption The other 19 patients had no signs of gastrointestinal disturbances

Patients 1–3 were given L-dopa as a solution in doses of 1 mg and 200 mg (Fig 1) 40 min before a standardized breakfast comprising two slices of bread and one glass of milk Patients 4–19 were given the L-dopa together with this standardized breakfast and this meal was also given patients 17–19 in connection with the loadings in the afternoon Patients 4–8 were given 500 mg L-dopa first as a solution and then as a rapid release tablet (Fig 2) Patients 9–17 were given 400 mg L-dopa as three kinds of tablet preparations i e rapid release sustained release and slow release tablets (Figs 3 4 5) Three patients (nos 17–19) had very marked diurnal variations in the clinical res

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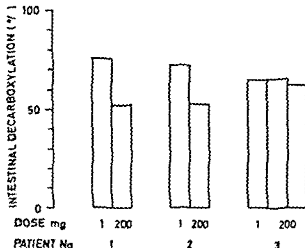


Fig 1 Degree of L-dopa decarboxylation in the intestinal tract after oral administration of 1 and 200 mg L-dopa dissolved in 150 ml water

to L-dopa administration The effect was rather good after the morning dose but was less satisfactory after the further L dopa doses during the day independent of whether the doses were taken with meals or not The intestinal decarboxylation was studied twice when 400 mg L dopa was given as rapid release tablets i e in the morning and in connection with the third L-dopa dose (Figs 6 7) Patient 20 was studied on four occasions when she received 400 mg L-dopa as rapid release tablets On the first two occasions the L-dopa was administered 20 min before isocaloric meals either rich in protein or carbohydrate On the last two occasions the L-dopa was given immediately after the same standardized meals

METHODS

The decarboxylation of L-dopa in the intestinal organs (in this paper called intestinal decarboxylation) was determined by a method previously described by Granerus et al (7) using ^{14}C labelled L-dopa (L 3 4-dihydroxy phenylalanine 1 ^{14}C) The fraction of the oral L-dopa dose (D) which was decarboxylated in the intestinal organs was calculated from the cumulative recovery in urine of ^{14}C activity after oral (Uo) and intravenous (Ui) loadings with carboxyl labelled L-dopa from the formula $D = 1 - Uo/Ui$ In addition to the oral loading an i v loading with carboxyl labelled L-dopa thus had to be performed in each patient The recovery in urine was corrected to a standard dose (25 μCi) when different amounts of radioactivity were given orally and i v

Unless otherwise stated the loadings were performed in the morning after fasting overnight with the patient in a sitting position The labelled compound was given orally as solutions (150 ml) containing 1 mg (specific activity 5 μCi/mol) 200 mg (specific activity 25 μCi/mol) and 500 mg (specific activity 10 μCi/mol) L-dopa and as tablets containing 400 mg (specific activity 12 μCi/mol) and 500 mg (specific activity 10 μCi/mol) L-dopa At the i v loadings 1 mg of the labelled L-dopa (specific activity 5 μCi/mol) was given in 10 ml saline as a rapid injection Half an hour before the injection unlabelled L-dopa was given orally in a dose corresponding to that given at the oral loading with labelled compound except for patients 1–3 who received only 200 mg unlabelled L-dopa

Urine was collected for three days after each loading After the administration of the labelled compound venous blood was drawn repeatedly in heparinized tubes during 6 hours in four patients the sampling time was extended over 24 hours In order to remove radioactivity originating from bicarbonate one volume of 4 M perchloric acid was added to 10 volumes of plasma or urine One ml of the supernatant obtained after centrifugation was added to 10

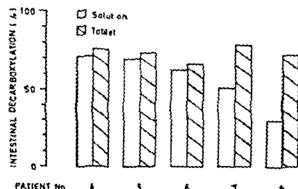


Fig 2 Degree of L-dopa decarboxylation in the intestinal tract after oral administration of 500 mg L-dopa dissolved in 150 ml water or given as a rapid release tablet

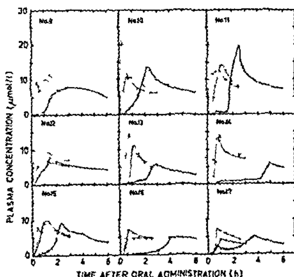


Fig 3 Plasma concentration of dopa plus non-decarboxylated dopa metabolites in 9 patients after oral administration of 400 mg L-dopa administered as three kinds of preparation (●- - -●=rapid release tablet ●—●= sustained release tablet ●——●=slow release tablet

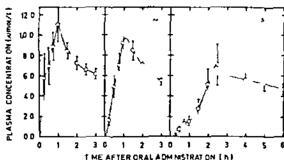


Fig 4 Concentration of L-dopa plus non-decarboxylated dopa metabolites in plasma (mean±S E $n=9$) after oral administration of 400 mg L-dopa administered as three kinds of preparation (rapid sustained and slow release tablets)

ml Instagel® and the ^{14}C activity originating from dopa 3 O methyldopa and other non-decarboxylated dopa metabolites was measured by a Packard Tri-Carb Liquid scintillation counter At least 20000 counts were registered which gave a statistical error of less than 1 4% (confidence level 95%)

Radioactive dopa and 3-O methyldopa were determined after ion exchange chromatographic separation (7) The purity of the radioactive material used (supplied by Hoffman La Roche Basel Switzerland for experiments in patients 1–8 and by Astra Sodertalje Sweden for experiments in patients 9–20) was checked by the same method More than 99% of the total radioactivity was found in the dopa fraction in all preparations

RESULTS

When L-dopa was given as a solution the intestinal decarboxylation was found to be around 70% of a given dose of 1 mg in patients 1–3 (Fig 1) In patient 3 a similar decarboxylation was also observed on two occasions when 200 mg L-dopa was given In patients 1 and 2 the intestinal decarboxylation was found to decrease to a mean of 50% when 200 mg was given When 500 mg L-dopa was administered as a solution to patients 4–8 (Fig 2) the decarboxylation in the intestinal tract amounted to a mean of 56 5% (S D ±16 8%) A comparison of the degree of intestinal decarboxylation when 500 mg was administered as a solution and as a rapid release tablet respectively was performed in patients 4–8 In all subjects the decarboxylation was higher when the L-dopa was given as a tablet (Fig 2)

When L-dopa was given as tablets of 400 mg with different resolvation times (patients 9–17) the time between tablet intake and the peak of the concentration in plasma did not differ significantly when rapid or sustained release tablets were given (Figs 3 4) but after intake of the slow release tablet the peak concentration was reached later There was no significant difference in the height of the peak concentration between the three kinds of tablets In patient 9 previously gastrectomized a high plasma concentration was obtained early after intake of a rapid release tablet but similar plasma concentration curves occurred in other patients

The degree of L dopa decarboxylation in the intestinal tract calculated from the urinary excretion of dopa plus non-decarboxylated dopa metabolites in these nine patients varied between 45% and 74% of the dose given (Fig 5) The mean decarboxylation after the rapid release tablet was 60 8% after the sustained release tablet 67 5% and after the slow release tablet 66 3% (Table I) The differences were not statistically significant (pairing desing test)

The three patients with a marked difference in clinical response to L-dopa during the day did not

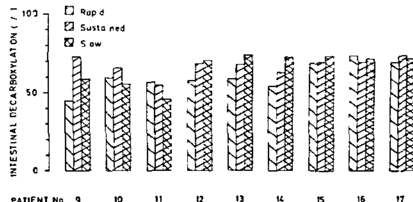


Fig 5 Degree of L-dopa decarboxylation in the intestinal tract after oral administration of 400 mg L-dopa administered as three kinds of preparation (rapid sustained and slow release tablets)

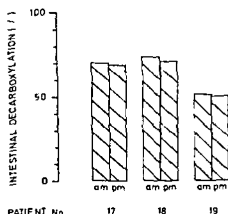


Fig 6 Degree of decarboxylation in the intestinal tract of orally administered L-dopa (400 mg rapid release tablet) in three patients studied under standardized conditions on two occasions On the first occasion the tablet was given in the morning as the first L-dopa dose and on the second occasion in the afternoon as the third L-dopa dose All three patients had complained of a successively less pronounced effect of each L-dopa dose during a day

show any difference either in the degree of L-dopa decarboxylation in the intestinal tract (Fig 6) or in the shape of the plasma concentration curve of dopa plus non-decarboxylated dopa metabolites (Fig 7) when the morning dose and the afternoon dose of L-dopa were compared

In the patient who was given L-dopa in connection with meals it was found that the degree of intestinal decarboxylation was the same regardless of whether L-dopa was administered 20 min before

Table I Intestinal L dopa decarboxylation and recovery in urine (mean±S D) of dopa plus non decarboxylated dopa metabolites (A) and of 3 O methyldopa (B) in nine parkinsonian patients after oral intake of 0 4 g L dopa as three kinds of tablets

	Intestinal decarboxylation (%)	Recovery in urine (%)	
		A	B
Rapid release tablet	60 8± 9 0	8 6±2 1	1 9±0 9
Sustained release tablet	67 5± 5 9	7 2±1 9	1 5±0 8
Slow release tablet	66 3±10 3	7 4±2 8	1 4±1 2
I v administration		*21 8±2 3*	*5 4±1 8*

a meal (63 64%) or immediately after a meal (64 70%) and also whether the meal was rich in protein (63 64%) or carbohydrate (64 70%) However if L dopa was given *immediately after a meal* there was a delay of several hours in reaching the maximal blood concentrations of L-dopa compared to when the drug was given before the meal

Evaluation of the method The intestinal decarboxylation as determined by the method described ought to be inversely correlated to the amount of dopa plus non-decarboxylated dopa metabolites which reaches the general circulation (here called net absorption) In four patients (nos 11 13 14 17) given three kinds of tablet prepara

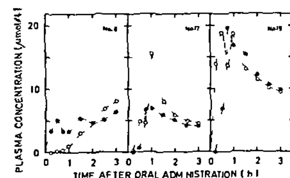


Fig 7 Plasma concentration of dopa plus non-decarboxylated dopa metabolites after oral administration of 400 mg L-dopa (rapid release tablet) in three patients studied under standardized conditions on two occasions On the first occasion the tablet was given in the morning (●---●) as the first L-dopa dose and on the second occasion in the afternoon (O---O) as the third dose All three patients had complained of a successively less pronounced effect of each L-dopa dose during a day

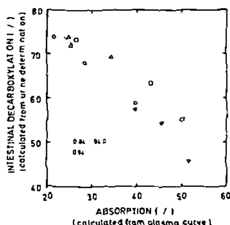


Fig 8 Correlation between the decarboxylation of L-dopa in the intestinal tract and the absorption calculated from the plasma concentration curve of dopa plus non decarboxylated dopa metabolites in the interval 0–24 hours after the administration of the drug in patients 11 (O) 13 (Δ) 14 (∇) and 17 (□) studied on three occasions

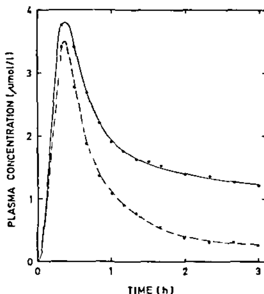


Fig 9 Plasma concentration of dopa (●---●) in relation to the total sum of dopa plus non-decarboxylated dopa metabolites (●—●) after oral administration of 400 mg L-dopa (patient 10)

tions the net absorption was calculated by comparing the area under the plasma curves in the interval 0–24 hours after the i v and the oral loadings respectively (Fig 8) As can be calculated from the regression line obtained an absorption of 50% and 30% respectively corresponded to an intestinal decarboxylation of 52% and 69% respectively

During the first 20 min after an oral loading with carboxyl labelled L dopa most of the radioactivity (>95%) in plasma was derived from dopa (Fig 9) when the activity originating from bicarbonate had been removed This shows that only negligible amounts of 3 O methyldopa and other non decarboxylated dopa metabolites were formed during the absorption and during the first passage through the liver

DISCUSSION

In the present study there was a close negative correlation between the method used for the calculation of intestinal decarboxylation and the net absorption calculated from the plasma concentration curve An intestinal decarboxylation of 50% corresponded to a net absorption of 52% as calculated from the regression equation and a decarboxylation of 70% to an absorption of 29% The sum of dopa and non-decarboxylated dopa metabolites was determined both by the calculation of intestinal decarboxylation from urinalyses and as the net absorption from plasma concentration The methods used could thus not disclose to what extent dopa was degraded to 3 O methyldopa or to other non-decarboxylated products in connection with the intestinal absorption and during the first passage through the liver However during the first phase of the absorption the radioactivity appearing in blood derived almost exclusively from dopa Thus the absorption calculated either indirectly from the intestinal decarboxylation or from the plasma concentration of dopa plus non-decarboxylated dopa *metabolites seems to represent the amount of* L-dopa that has reached the general circulation

The amino acid L dopa is pharmacologically an inert compound in itself The pharmacological effects seen after L-dopa administration are mainly caused by the decarboxylated products formed e g dopamine (3) Unlike L-dopa the decarboxylated products do not readily pass the blood brain barrier and consequently the peripherally produced dopamine will not be available for the central dopaminergic neurons Furthermore the peripherally produced dopamine will cause some side effects of the L-dopa therapy (1 5)

Orally given L dopa is absorbed to only a small extent as unchanged L dopa as a large part is decarboxylated already in the intestinal organs (2 7) The decarboxylation possibly takes place both in the liver and the intestine as decarboxylase activity has been found in both these organs (4) It has been indicated that histidine can be decarboxylated to histamine in the upper part of the gastrointestinal tract by the action of bacteria (8) A decarboxylation of L-dopa within the gastrointestinal lumen therefore also has to be taken into consideration This has also been suggested by Sandler et al (11)

The absorption of the dietary amino acids appears normally to be very efficient Earlier studies have shown that L-dopa inhibits the absorption of L phenylalanine (7) which indicates that these amino acids share a common transport mechanism The present study indicates that L dopa also is easily absorbed although only a smaller part reaches the general circulation due to a high degree of decarboxylation in connection with the absorption or the passage through the liver

A striking observation was that about 50–70% of orally administered L dopa was decarboxylated in the intestinal organs whether given as a solution or

as tablets with different resolvation times and whether given as a tracer dose of 1 mg or as conventional doses of 200–500 mg Neither did an ordinary meal influence the degree of decarboxylation The overall bioavailability of orally administered L-dopa therefore seems to be rather independent of dose and kind of pharmacological preparation As expected and as earlier shown by Rinne et al (10) the plasma concentration curve of dopa is influenced by the kind of pharmacological preparation given In the present study it was shown that delayed absorption of L dopa could be achieved both by using a slow release tablet and by administering L-dopa immediately after an ordinary meal A delay of 2–4 hours in reaching the maximal L-dopa concentration in the blood was observed This could be of clinical significance in situations where there is an indication to reduce the number of L-dopa doses or to even out the fluctuation of circulating dopa in plasma which possibly could be associated with fluctuations in the clinical response (12) and to the appearance of side-effects However a delay in reaching therapeutical concentrations in plasma when L-dopa is given together with food could be misinterpreted either as a lack of response to a certain L-dopa dose or as an effect of overdosage if there is an additive effect of two L-dopa doses It should be noted that a rather high concentration in plasma could be obtained even when slow release tablets are used or when L-dopa is administered after a meal

The intraindividual variations in the shape of the lasma concentration curves after repeated administration under standardized conditions during the day were rather small while the interindividual differences generally were marked The variations in clinical effect in the morning and later during the day did not seem to be related to any obvious differences in L dopa absorption

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Survival of Patients on Hemodialysis after Renal Graft Failure

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ABSTRACT The influence of kidney graft failure on the survival rate after reentering maintenance hemodialysis has been studied in 63 patients Twenty eight patients were observed for at least 12 months after a primary graft failure and 35 had a second transplant some time during this period Patient survival 12 months after graft failure was 57 % This result was compared with the 1 year survival of 82 % in 150 patients dialyzed prior to the first transplantation The majority of the deaths on dialysis after graft failure were caused by infectious complications probably related to the immunosuppressive treatment It is suggested that survival on dialysis after graft failure may be improved by an early reduction of the immunosuppressive treatment in patients with failing grafts

During the last few years dialysis and kidney transplantation have yielded only a very slow improvement in the survival of patients with terminal uremia (1) The best long term results have been reported in patients on home dialysis and in recipients of related living donor grafts (6) However equally good results have also been achieved in patients waiting for renal transplantation on center dialysis as on home dialysis (10) The constant shortage of dialysis facilities has often made it necessary to enter an integrated dialysis and transplantation program to take care of the uremic patients suitable for treatment Also with a high rate of transplantation there are many patients who need maintenance hemodialysis for long periods due to the difficulties of for instance finding a suitable graft In other patients transplantation of a kidney is only a temporary success since the graft may be rejected after varying periods These patients have to reenter dialysis treatment and may later have a second transplantation

The gradual expansion of treatment facilities has made it possible to offer some degree of choice between maintenance dialysis and transplantation It has therefore become of interest and importance to evaluate whether resumed chronic maintenance hemodialysis after a graft failure carries a greater mortality risk than dialysis before transplantation and to compare the prognosis on hemodialysis treatment after a graft failure with the risks involved in a second attempt at transplantation

MATERIAL

The investigation was carried out on patients treated in Sahlgren s Hospital University of Goteborg Sweden and two co-operating hospitals In these hospitals the management of patients with terminal uremia has changed very little over the years and has been reported before (2 7)

The recipients considered for treatment were with very few exceptions accepted both for dialysis and for transplantation but with the program aimed ultimately at transplantation Recipients aged 10–60 years were accepted in many instances with complicating extrarenal disorders Since 1969 the selection of recipients was done according to HL A match (13) This has resulted in a considerable accumulation of patients with infrequent HL A antigen combinations particularly within ABO blood group 0 (7) Patients who developed circulating cytotoxic HL A antibodies very often had to wait a relatively long time for a suitable graft In some instances a patient on hemodialysis with severe problems of access to the bloodstream psycho-social disturbance or other complicating factors was accorded priority for an early transplantation These factors certainly involved some degree of recipient selection but this was not considered to impair the conclusions at all substantially

cal situation for the second graft In our experience there is no difference between patient survival after first and second transplantation

Patient survival after first graft failure may thus be improved by early retransplantation or a prompt return to dialysis treatment rather than continued immunosuppressive therapy which could lead to serious complications The policy of retransplanta tion is particularly applicable to patients in whom the rejection process has been slow and chronic and in whom the high initial amounts of immunosuppressive therapy have been tapered to a safe maintenance therapy Patients who on the other hand have experienced a rapid progression towards graft failure shortly after transplantation and to whom a vigorous immunosuppressive therapy has been applied may benefit from an early withdrawal of therapy pressure and subsequent removal of the graft

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A Case of Disseminated Hemangiomatosis with Cutaneous, Hepatic and Skeletal Manifestations and Increased Urinary Excretion of Glycosaminoglycans

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ABSTRACT A case of disseminated hemangiomatosis with cutaneous, hepatic and extensive skeletal manifestations is reported The diagnosis was established by means of bone X ray, hepatic angiography, and skin and bone biopsies A moderately increased urinary excretion of glycosaminoglycans (GAG) was found The main GAG fraction excreted was tentatively identified as a heparan sulphate

Isolated hemangiomas occur frequently in the skin and in the bones they are found in about 10% of all autopsies (11) Isolated hemangiomas may also occur in subcutis brain and visceral organs (13 16) The disseminated form of hemangiomatosis is rare however and often poses difficult diagnostic problems Most cases reported have been diagnosed only during surgery or autopsy (13 16) Disseminated hemangiomatosis can be divided into two categories hereditary hemorrhagic teleangiectasia occurring in childhood and hemangiomatosis occurring in adults Hemangiomas may be either capillary or cavernous occasionally both types are present simultaneously Usually they are benign tumors but malignant hemangiosarcomas occasionally occur in adults

Wallis et al (16) have reviewed the cases of disseminated hemangiomatosis described up to 1964 and introduced the following classification diffuse skeletal hemangiomatosis *a*) with visceral involvement and *b*) without visceral involvement Their series consisted of 9 cases in group *b* all collected from the literature and 17 cases in group *a* including 2 of their own They further subdivided those cases with visceral involvement into histologically benign (13 cases of 17) and histologically malignant (4 cases of 17) To the best of our knowledge only 2 further cases belonging to the group with visceral involvement have been described since then (3 15)

In the present paper a case of disseminated hemangiomatosis with cutaneous visceral and skeletal involvement is described

CASE REPORT

The patient is a 51 year-old engineer There was no hereditary history of hemangiomatosis In 1964 he had icterus due to an unknown case for a few weeks In 197 a Warthin s tumor (cystadenoma papillare lymphadenomatosum) was excised from the neck No malignant features were observed Since 1964 he had noticed small naevi 3–4 mm in diameter appearing in the face neck and upper part of the trunk and once on the mucous membrane of the nose Because of profuse bleeding about two to five of them had to be excised annually The histological picture was always that of capillary hemangioma

In the beginning of 1972 an i v urography was performed due to an attack of uretherolithiasis The kidneys were normal but in the lumbal spine sacrum and pelvis multiple lesions were seen and based on these X ray findings the diagnosis of Paget s disease was introduced

During a routine physical examination the liver was found to be markedly enlarged and nodular The patient was referred to our department for further examination in March 1974

The patient was apparently in good health Physical examination revealed two hemangiomas in the face (about 3 mm in diameter) and an enlarged nodular liver which reached 10 cm under the costal margin Neither circulatory bruits nor signs of circulatory failure were detected The fundi of the eyes were normal

Radiological findings

Skeletal involvement was detected in the following locations the skull thoracal and lumbal spine proximal

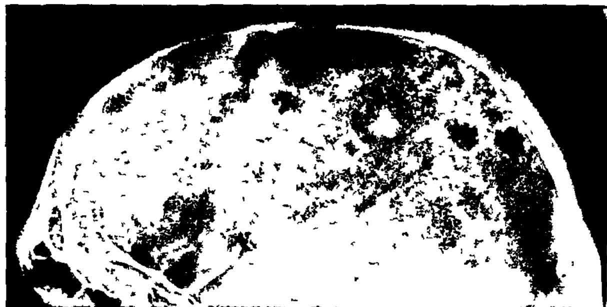


Fig 1 Skull with well defined demineralized areas in the parietal region The borders of the lesions are sclerotic as is part of the bone between them

of the humeri and femora pelvis and ribs 8 to the right and 7 and 11 to the left The vertebral lesions consisted of sclerosis and loss of trabecular structure of the vertebral bodies The lesions in other parts of the skeleton consisted of well defined partly osteolytic partly sclerotic areas with a typical honeycomb or sunburst appearance (Figs 1 2)

An arteriography showed no hypervascularity of the in the pelvis A celiac arteriography revealed a enlarged liver with a common hepatic artery of normal width but tortuous and dislocated intrahepatic arteries A filling of numerous lacunae in the parenchyma of the liver was seen (Fig 3 a b) Filling of the lacunae began 2 sec after the injection of contrast medium had started The arterial circulation time of the liver from the end of the injection until the lacunae emptied of contrast medium was 12 sec Thus no signs of shunting of blood through the liver could be detected The portal vein was normal but the intrahepatic branches were dislocated in the same fashion as the arteries The spleen did not show



Fig 2 Pelvis and hips with well defined demineralized lesions with sclerotic margins and sparse sclerotic trabeculae One of the lesions in the left iliac bone (→) has the characteristic sunburst appearance of a hemangioma of the bone



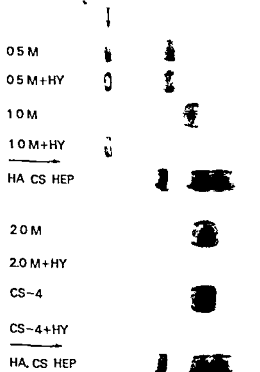


Fig 3 (*a*) Arterial phase of a celiac angiography where tortuous and d slocated intrahepatic arteries are seen (*b*) Parenchymal phase of the same invest gat on with the fill ng of cavernous lesions spread throughout the paren chyma of the liver

any hemangiomatous involvement Cholecystography re vealed multiple gallstones The liver scan showed in accordance with the angiography a markedly enlarged liver with multiple nodular defects The γ scann ng of the brain showed multiple hyperactive sites at the frontal parietal region

Laboratory find ngs

The ESR was 40–50 mm/h but in other respect he blood picture was normal Bleed ng and coagulat on t me were normal Serum haptoglobin concentrat on and ran aminases were sl ghtly elevated The retention brom sulphtale n (BSP) was 9% after 45 min norm alue



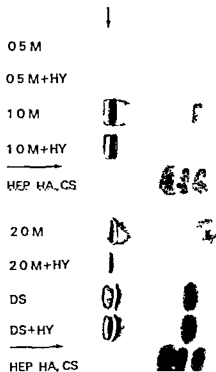


Fig 4 Electrophoretic behavior of the various urinary GAG fract ons and GAG standards in pyrid ne form c acid pH 3 0 (left) and in 0 1 M barium acetate (right) The starting points are indicated by arrows *HY* hyaluronidase *HA* hyaluronate *CS*–chondroitin sulphate *HEP*=heparin *DS*=dermatan sulphate

Since no patient with diffuse hemangiomatosis limited to the skeleton seems to have died of this disease it is likely that the clearest prognostic criterion is offered by the degree of visceral involvement (13 16) If intrathoracic structures pleura or lungs are grossly involved the prognosis seems to be worse Patients with visceral involvement have lived 1–12 years after the onset of the disease (16)

Vascular tissue has a relatively high content of GAG (1–2% of the dry weight) From mammalian arterial tissue hyaluronate chondroitin-4 & 6 sulphate dermatan sulphate and heparan sulphate have been isolated (2) It appears reasonable to assume that in diffuse hemangiomatosis the increased body content of vascular tissue and hence also of many species of GAG could be associated with an increased urinary excretion of these compounds or their derivatives According to the preliminary findings reported here this assumption seems correct

It is interesting that the increased excretion of GAG appears to be due to an increased excretion of heparan sulphate derivatives whereas the excretion of chondroitin sulphates normally the main components of the urinary GAG falls within normal limits Further analytical studies are necessary however to establish the existence of a qualitative deviation in urinary GAG excretion in diffuse hemangiomatosis

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LETTER TO THE EDITOR

Sir

I have read and reread Dr Gunnar Biorck s thought provoking editorial on page 441 in the July issue of 1974 and I feel that it needs an answer Some of Dr Biorck s thoughts are correct but the underlying principle and in particular his inferences are wrong

I agree with the editorial that preventive health screening is not going to solve all the problems of medicine It may make a significant contribution to part of those problems and that in itself should be sufficient to grant it a place But continuing on from there the editorial draws some strange conclusions I am sure that many people would hope to die suddenly and without warning as was suggested by an inquiry in a Swedish magazine Unfortunately the editorial does not say when and I am sure that the majority would like to die at a ripe old age In this respect I refer to today s obituaries in the New York Times Of the 13 people written up five died at ages 63 or below which is generally considered too young Part of the purpose of preventive health screening is to prevent death in this range

My major objection however is that the author assumes that society and the collection of its individuals have different interests This is a fallacy If each individual wishes to live to 90 no matter what the consequences then we all want to live to 90 and by definition society wants to live to 90 For verification visit an old age home Go around with a list and offer to give people a needle so that to use the words of the editorial they would pass away suddenly and without warning Very few people would sign a release

The reason for this discrepancy is that we who are over a certain age sometimes project our own desires on those of another age People in their 20 s would not like to be 50 because 50-year-olds may have certain restrictions in their way of life But once they become 50 they may discover certain compensations which they were not aware of before The same thing happens when a 50-year-old projects himself on the age of 80 It would therefore be wrong for those of us who are 20 or 40 or 60 to decide if the 80- and 90-year-olds want to live That should be left up to them If they want to continue living then we may assume that once we reach that age we should want to do likewise And therefore if all people that make up society would want to live once they are that old there is no reason for society to decide that they should not And fearing as is done in the article that preventive medicine may not be a good thing is therefore wrong by definition

It has always been the task of physicians public health engineers and all their assistants to provide the largest amount of health the least amount of sickness and the longest life possible It would be inappropriate to exclude preventive health screening simply because we who are younger believe that they who are older have nothing interesting to live for

Yours very truly
E de Haas Princeton USA

Dear Dr de Haas

Professor Waldenstrom has forwarded your letter to me

I agree with most of what you say concerning people s wishing to live on further at almost any age Journey s end gets postponed when you arrive at an age which you formerly considered to be enough There is no plea or proposal whatsoever in my editorial implying any change in society s care of the aged But it is precisely in this respect that we who are the advocates of the old and infirm to-day find ourselves in trouble because their numbers increase so fast and their need of institutional care increases even more so that the political bodies of the present tax payers (who are at the same time the decision makers because older people do not get elected to political offices nowadays which means that perhaps the older 15% of the population are not represented in Parliament) hesitate to pay the bill Today a politician said in the Swedish Medical Journal we must provide care at the lowest possible level because it is a concern to the tax payers Then they turn their minds towards prevention and become seduced by the idea that health can be bought at a much cheaper price than care and this illusion is further strengthened by medical economists who present calculations on savings in manpower and similar things at the same time completely disregarding the acute needs of people of retirement age

To my mind in my country—I admit that bio-social development takes place at different rates in different countries—there is little doubt that the care of the chronically sick and disabled older citizens is *the* dominating

medical problem because it seems impossible to create sufficient resources particularly as regards (wo)man power and finance for the proper care of this ever growing group I do not think that preventive medicine can prevent (but possibly postpone) the accumulation of sick and disabled persons at life s farther end It will most probably leave this problem as unsolved as today

Speaking of obituaries I made the observation the other day that among roundabout 30 death announcements in one daily newspaper eight were signed also by great grandchildren No doubt sooner or later we will find one signed by great great grandchildren We *are* in the process of becoming a four generation society and we will eventu ally arrive at a five generation society It is easy to con ceive of members of two generations being admitted to a hospital for the chronically ill and eventually this might apply to even three generations If science is the knowledge of consequences we as medical men must reflect on the ultimate results of our endeavours Some of these results may be seen in overpopulated countries in the third world Others may be recognized in the ar rangements we make—or do not make—for our two or three eldest generations

Gunnar Biorck

BOOK REVIEW

Tumors of the urinary bladder By Leopold G Koss 120 pages US $8 Armed Forces Institutes of Pathology Washington D C USA 1975

The Atlas of Tumor Pathology started to appear in 1947 This second series of the Atlas is not intended as a second edition It retains the basic purpose of providing an atlas setting standards of diagnosis and terminology A broader perspective of tumors is offered by the inclusion of special stains histochemical illustrations and electromicrographs with data on biologic behavior Special attention is paid to the grading of malignant tumors usually from I to IV to express the degree of histologic and cytologic abnormalities This is highly essential in view of the relation between stage grading prognosis and choice of therapy for these very complicated tumors There is an excellent review of different kinds of tumors both malignant and benign forms and also of therapy induced changes in histology and cytology

For all specialists working with the diagnosis and treatment of bladder tumors in general this tumor atlas *will be of the greatest interest*

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EDITORIAL

Preleukemia

It is becoming possible to predict the development of myeloblastic leukemia in an increasing number of hematologic conditions (5 14 27 32) In those developing spontaneously no effective preventive measures are known so far However attempts to develop such measures may perhaps be stimulated if the problem is defined and recognized

Prevention becomes even more important if some preleukemic conditions are iatrogenic Further studies of the possible causative role of chemotherapy and medically used radiation are required to establish if there are really relevant iatrogenic forms Such studies will show if it will become necessary to weigh the indications for chemotherapy or repeated X ray examinations against the possible risk of inducing leukemia

Clinical features of preleukemia

A large number of more or less specific hematologic changes have been observed to precede leukemia The best predicting value may be found in the initially apparently non neoplastic cases in a previously described combination of anemia hyperplastic bone marrow sections and an increase in the number of intermediate sideroblasts The whole body number of red cell precursors in these patients is not increased it is myeloid hyperplasia which explains the hypercellularity (5 24 25 27 29 30)

The patients with this preleukemic syndrome have been shown to have decreased bone marrow cell proliferation rates i e significantly increased generation times (29) as is the case in manifest leukemia (17) These patients also have significantly decreased ^{3}H thymidine bone marrow cell labelling indices (29 30)

In the patients with the non neoplastic non sideroblastic hypoplastic form of aregenerative anemia generation times and labelling indices are normal

The predicting value has been less well studied of the Pelger Huet abnormality (5) large platelets (27) decreased leucocyte alkaline phosphatases and decreased phagocytic capacity (3) sometimes seen

Chromosomal aberrations of many non specific kinds have been described (23 31) However abnormality particularly aneuploidy of the group C-chromosomes appears to be associated with disturbances of hemopoiesis This has been found both in myeloproliferative diseases (30) and in a few patients with preleukemia (23) Both missing and extra C chromosomes have been found and it has been suggested that this genome while not producing neoplasia itself may provide a favorable milieu for some transforming agent (23)

Initially apparently non neoplastic conditions

Several apparently initially benign usually chronic cytopenias seem to be of a preleukemic nature Most common among these seems to be one form of chronic refractory hypoproliferative or aregenerative anemia This form of anemia is accompanied by hypercellular or hyperplastic bone marrow sections with a reduced fat content (24 25 28) These patients have significantly more intermediate sideroblasts than normal subjects i e erythroblasts with more than 7 stainable iron grains but no ring form arrangement (10 11 12 24 25) A number of other hematological less regular findings are described below This form of anemia may be distinct from a similarly chronic refractory form with hypoplastic fatty bone marrow sections and no increase in the number of intermediate sideroblasts

Out of 18 such patients with hypercellular mar rows who could be followed to autopsy 14 (78%) died with a typical picture of acute leukemia as compared to only 2 out of 23 patients (9%) with hypocellular marrows (7 24 25) It appears there fore that despite fairly non specific initial findings leukemia can be predicted with some degree of accuracy in patients with chronic refractory aregenerative anemias

Less common but better defined and with more specific initial symptoms are the preleukemias in paroxysmal nocturnal hemoglobinuria (PNH) (13 33) and the anemias with ring formed sideroblasts (4) PNH like the aregenerative anemias may have granulocytopenia (44% of the patients) thrombocytopenia (65%) and hypoplastic bone marrows (13%) PNH may be a somatic mutation (13) possibly arising more easily in hypoplastic or fibrotic bone marrows (15) The most common causes of death are thromboses (5 out of 9 cases) infections anemia and bleeding I have found no figure for the frequency of leukemia which may perhaps be no higher than in the above mentioned aregenerative anemias with hypoplastic marrows which do not have hemoglobinuria (9%)

Iatrogenic leukemia has been demonstrated in patients given radiotherapy for ankylosing spondylitis (9) or other causes

Initially neoplastic conditions Iatrogenic contributory causes of preleukemia

A hematologic sometimes premalignant condition which is not of a preleukemic nature is *benign monoclonal gammopathy* However in one study only about 4% of these patients developed myeloma during a 6-year period of observation

It is well known that about 70% of patients with chronic myelocytic leukemias (CML) develop a so-called blastic crisis which resembles acute leukemia and it has been suggested that CML could regarded as a preleukemic condition (17) In the terminal stages of myelofibrosis and of polycythemia vera pictures resembling acute leukemia are also sometimes seen (8 19 20 21) The development of leukemia in these patients could be a part of the natural course of their primary disease but long term treatment with cytostatic radiomimetic and potentially leukemogenic drugs might contribute

Modan (19) and Modan and Lilienfeldt (20 21) have demonstrated that the incidence of acute leukemia in the terminal stage of polycythemia vera is almost 10 times as high when ³²P treatment is given as when more conservative therapy is used although other complications are more frequent in the latter group Here iatrogenic preleukemia may be considered certain although the primary disease contributes some leukemias develop even in the absence of ³²P or myleran treatment

In myeloma the picture is less clear At least 23 patients with melphalan treated myeloma developing into myeloblastic leukemia have been described (1 18 33) Of the 23 21 had received continuous melphalan treatment whereas 2 had received intermittent treatment (18) At least 97 patients who developed leukemia after a lymphoma treated with alkylating drugs have also been described Eighty five developed acute and 12 chronic myeloid leukemia (2 6 16 18 26) Most of the patients had received radiotherapy—the most likely cause of the leukemia—many had both radio and chemotherapy but a few leukemias developed after chemotherapy alone Occasionally we have observed that manifest leukemia in such patients may be preceded by anemia and a hyperplastic sideroblastic marrow just as in the preleukemic form of aregenerative anemia

There are thus indications that the frequency of leukemia in myeloma may have increased after the introduction of alkylating agents The question is if the alkylators are leukemogenic or if these drugs prolong survival and thus make it possible for leukemia to appear It seems probable that they are leukemogenic since alkylating drugs have been followed by myeloblastic leukemia even in conditions in which leukemia appears less likely to be a part of the natural history of the disease than in myeloma such as cancer of the ovary bronchi breast (22) or other organs (34)

The possible leukemogenic effect of diagnostic radiation is still controversial but it is of particular interest because of the extensive use of X ray examinations to-day About 9 mill X ray examinations per year are performed in Sweden—more than one per inhabitant There are indications that in men the risk of developing acute or chronic myelocytic leukemia is increased 5 3 times when minor or major radiologic examinations are repeated 41 times or more (13) If only major examinations are done the risk increases 12 2 times

It is important to establish the natural course of

preleukemia so that the possible effect of drugs and radiation may be established and reduced Further studies of preleukemic cells could also contribute to the understanding of the cellular defect in manifest leukemia It would be interesting if for instance reverse transcriptase or vira could be found during the preleukemic stage

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BOOK REVIEW

Biochemistry By Lubert Stryer Yale University 877 pages ISBN 0-7167-0174 X $19 95 W H Freeman and *Company San Francisco 1975*

For the modern clinician access to an up to date textbook on biochemistry is a must The rapid movement of the frontiers in this subject is one great difficulty both for author and reader and the fact that clinical medicine becomes increasingly involved in problems at the molecular level makes a chemical outlook imperative The number of diseases caused by sick molecules is ever increasing and curiously enough some of them are amenable to treatment even if they are genetically determined and therefore are examples of unescapable predestination in the individual patient

This work is outstanding It gives the latest facts and quotes the last publications—well chosen This is of course necessary for a book on a subject that is developing all the time The illustrations are first rate with a wealth of material and lavish use of colour that helps the reader to grasp even complicated molecular structures at a glance The organization of the material and its typographical presentation are ideal also for the clinically oriented reader

The author's own contributions have been largely in the field of nucleic acids and these difficult chapters are very well written They convey the complicated facts in a clear presentation The reviewer has old and special interests in the porphyrias My personal opinion is very definitely that the clinical parts in this chapter should be differently disposed Congenital porphyria that is one of the rarest diseases in the world (probably about 25 cases described since the first in 1897) fills a large part of the clinical pages whereas other really important diseases are not treated It would have been better to leave out the science fiction story about George III Forget it!

The metabolism of what the Germans call Wirkstoffe active substances "biomolecules such as steroid and pituitary hormones biogenic amines (histamine 5 HT) polypeptide intestinal hormones etc may perhaps be discussed at greater length in a coming edition

On the whole this splendid volume may be recommended both as a textbook for advanced students of medicine and as a reference work for hospitals and laboratories

Jan G Waldenström

Acta med scand Vol 198 pp 437–443 1975

Plasma Myeloperoxidase and Lactoferrin Measured by Radioimmunoassay Relations to Neutrophil Kinetics

Niels Ebbe Hansen Jörgen Malmquist and Jan Thorell

From Division of Hematology Department of Medicine A Rigshospitalet University Hospital Copenhagen Denmark and Departments of Medicine and Nuclear Medicine University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT In 31 patients covering a wide range of blood neutrophil counts and turnover rates, the plasma concentrations of myeloperoxidase and lactoferrin have been measured with radioimmunoassays and compared to neutrophil kinetic parameters, measured with DF^{32}P labeled neutrophils It was found that the plasma concentrations of both proteins correlated significantly with the total number of neutrophils in the blood (TBGP=total blood granulocyte pool) as well as with the neutrophil turnover rate (GTR=granulocyte turnover rate), which is evidence that neutrophilic granulocytes are the main suppliers of myeloperoxidase and lactoferrin to the plasma In contrast to the previously demonstrated better relationship between the GTR and plasma lysozyme, a protein also originating in neutrophil granules, both myeloperoxidase and lactoferrin correlated better with the TBGP These differences may reflect differences in the mode of release of intragranular proteins from neutrophils to the plasma The correlation of the plasma lactoferrin concentration with the TBGP was so good as to suggest its use in the clinical assessment of the TBGP

The relationship between neutrophil kinetics and the plasma concentration of proteins presumably originating in neutrophilic granulocytes is of interest partly because the source and mechanisms of release into the plasma of the proteins in question may be elucidated and partly because—if a significant correlation is demonstrated—determination of the plasma protein concentrations might be used as a clinical tool in the assessment of neutrophil kinetics

Previously the relationship between neutrophil kinetics and the serum or plasma concentrations of vitamin B_{12} binding protein and lysozyme has been studied Thus Chikkappa et al (5) demonstrated that the serum concentration of B_{12}-binding protein correlates with the total number of neutrophilic granulocytes in the blood possibly because this protein is continuously released from intact cells in the circulation whereas the plasma lysozyme concentration was found to correlate with the neutrophil turnover rate presumably because this enzyme is released to the plasma from disintegrating neutrophils in the tissues (9)

It is the aim of the present study to investigate the relationship between neutrophil kinetic data and the plasma concentrations of myeloperoxidase and lactoferrin Like lysozyme these proteins are believed to play a major part in the defense against bacteria and also like lysozyme they are localized in the granules of the neutrophilic granulocytes Whereas myeloperoxidase occurs in the primary (azurophilic) granules which are formed at the promyelocyte stage lactoferrin is found in the secondary (specific) granules formed at the myelocyte stage lysozyme is found in both types of granules Although this granular distribution of the three proteins refers to findings in the rabbit which is the species most extensively studied these main differences in the granular distribution of the three proteins also apply to human neutrophils even though the granular pattern may be more complex in humans than in rabbits (1 2 4 7 12 16 25)

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Table I *Clinical kinetic and plasma protein data in 9 patients with normal neutrophil counts*

Pat no	Diagnosis	Neutrophils (per μl)	TBGP (10^7/kg)	GTR (10^7/kg/d)	Myeloperoxidase (μg/ml)	Lactoferrin (μg/ml)
1	Pernicious anemia (untreated)	2 640	60	132	0 5	0 2
2	Pernicious anemia (untreated)	2 914	42	100	0 5	0 2
3	Chronic granulomatous disease	2 532	63	213	0 6	1 1
4	Preleukemia	2 900	97	116	0 4	1 0
5	Hodgkin s disease	6 350	112	304	1 0	0 5
Hematologically normal patients						
6	Myalgia	2 492	50	123	0 4	0 2
7	Duodenal ulcer	3 835	56	153	0 5	0 7
8	Serous coxitis	4 727	76	188	0 6	0 5
9	Prolapse of vertebral disc	2 254	57	148	0 4	0 3
Normal values			27–138	62–400	0 3–0 5	0 13–0 42

MATERIAL AND METHODS

The material consists of 31 kinetic studies in 29 patients previously reported in a study on the relationship between plasma lysozyme and neutrophil kinetics (9) All the patients had normal serum creatinine levels and were free from infection Relevant clinical data are shown in Tables I–III

Kinetic studies

Neutrophil kinetic studies were performed with radioactive diisopropylfluorophosphate ($DF^{32}P$) as previously described (9) The two most important parameters from these kinetic studies are the total number of neutrophilic granulocytes in the blood stream (TBGP=total blood granulocyte pool) calculated with an isotope dilution principle and the neutrophil turnover rate GTR= granulocyte turnover rate) calculated from the TBGP and the blood disappearance half time of labeled neutrophils (T/2) GTR=TBGP×ln 2/T/2

Myeloperoxidase and lactoferrin assays

EDTA plasma samples were stored at −18 C until analysis The proteins were measured by radioimmunoassays using the following reagents and procedures

The purification of myeloperoxidase and lactoferrin and the preparation of specific rabbit antisera were performed as described previously (14 15) Myeloperoxidase was iodinated with ^{125}I by means of polyacrylamide lactoperoxidase (23) Unreacted iodide was removed by dialysis The specific activity was about 8 μCi/μg corresponding to 0 5 mol I/mol protein The labeled protein was stored at −20°C in 2% bovine serum albumin For use in the assay it was diluted to 20 ng/ml ^{125}I lactoferrin was prepared with lactoperoxidase (22) Unreacted iodide was removed by dialysis The specific activity was about 18 μCi/μg corresponding to a substitution of 0 7 mol I/mol protein Storage and dilution for assay were as for ^{125}I myeloperoxidase The myeloperoxidase antiserum was diluted 1 6400 and the lactoferrin antiserum 1 12800 In the assays 0 05 ml of these dilutions were used

Both proteins were assayed by double antibody radioimmunoassay according to identical protocols Barbital buffer 0 075 M containing 0 25% bovine serum albumin was used as diluent for all reagents

Each tube of the assays contained 0 1 ml standard (0–0 2μg) or plasma sample 0 05 ml ^{125}I labeled protein 0 05 ml antiserum dilution and 0 2 ml diluent The tubes were incubated overnight at 4 C The antibodies were then precipitated by adding 0 05 ml goat anti rabbit

Table II *Clinical kinetic and plasma protein data in 9 patients with low neutrophil counts*
Normal values see Table I

Pat no	Diagnosis	Neutrophils (per μl)	TBGP (10^7/kg)	GTR (10^7/kg/d)	Myeloperoxidase (μg/ml)	Lactoferrin (μg/ml)
10	Pancytopenia preleukemia	136	5	9	1 6	0 5
11	Pancytopenia preleukemia	1 015	22	181	0 4	0 6
12	Aplastic anemia	278	3	87	0 5	0 1
13	Chronic neutropenia	360	8	42	0 5	0 1
14	Pernicious anemia (untreated)	966	19	43	0 4	1 3
15	Lymphosarcoma (cyclophosphamide treated)	1 193	34	55	0 5	0 4
16	Hodgkin s disease (irradiated)	1 650	19	27	0 3	0 1
17	Cirrhosis of the liver splenomegaly	918	58	91	0 3	0 6
18	Cirrhosis of the liver splenomegaly	1 662	63	508	0 5	0 1

Table III *Clinical kinetic and plasma protein data in 13 patients with high neutrophil counts*
CML chronic myelocytic leukemia Normal values see Table I

Pat no	Diagnosis	Neutrophils (per μl)	TBGP (10 /kg)	GTR (10 /kg/d)	Myeloperoxidase (μg/ml)	Lactoferrin (μg/ml)
19	Rheumatoid arthritis prednisone treated	8 013	207	171	0.3	0.9
20	Lymphosarcoma	11 056	270	383	0.5	1.1
21	Lymphosarcoma	16 611	281	441	0.9	1.0
22	Hemolytic anemia (G 6PD deficiency)	17 558	193	448	1.1	1.7
23	Myelofibrosis	8 757	650	539	1.2	1.6
24	Polycythemia vera	15 738	227	393	0.7	1.0
25	Polycythemia vera	22 295	1 043	1 073	0.5	1.1
26	Polycythemia vera	9 417	268	748	0.7	1.5
27	Polycythemia vera	38 180	767	771	1.2	1.6
28	CML	26 033	242	404	1.2	1.9
29	CML	83 680	1 331	323	1.4	3.4
30	CML	327 360	8 005	1 826	8.9	9.0
31	Acute myeloid leukemia	151 200	2 260	737	2.9	5.3

γ globulin antiserum (1 10 dilution) and 0.05 ml normal rabbit serum (1 250 dilution) To compensate for plasma effects in the precipitation 0.1 ml normal human plasma was added to the standard tubes and 0.1 ml diluent to the sample tubes The contents of myeloperoxidase or lactoferrin of this plasma added just before the precipitation were shown not to influence the binding between the radioligand and antibody in the primary reaction

The tubes were incubated for another 20 hours 0.5 ml diluent was then added to each tube followed by centrifugation at 2500 *g* for 15 min The supernatant was decanted and the precipitate was counted in an automated well counter

RESULTS

Representative standard curves for the radioimmunoassays are shown in Fig 1 As the protein concentrations to be measured were on a high level *for a radioimmunoassay no sensitivity problem* was encountered

A provisional normal range for plasma myeloperoxidase was established with samples from 10 healthy laboratory employees The range found was 0.3–0.5 μg/ml

The plasma lactoferrin in 49 blood donor samples was found to be 0.13–0.42 μg/ml

The neutrophil data and the plasma concentrations of myeloperoxidase and lactoferrin from each study are shown in Tables I III

Figs 2 and 3 show the relationship between plasma myeloperoxidase and lactoferrin and the kinetic parameters TBGP and GTR It is seen that both proteins correlated significantly with both the TBGP and the GTR although both proteins correlated better with the TBGP It is also seen from these figures that plasma lactoferrin correlated better with both kinetic parameters than did myeloperoxidase and that the correlation between plasma lactoferrin and the TBGP was excellent Because the data did not conform with a normal distribution mode statistical analysis was performed after log transformation of the data

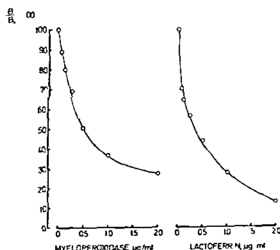


Fig 1 Standard curves for the radioimmunoassays *Abscissa* concentration of protein standards *Ordinate* the amount of antibody bound labeled antigen (*B*) expressed as percentage of the binding in the absence of unlabeled antigen (B_0)

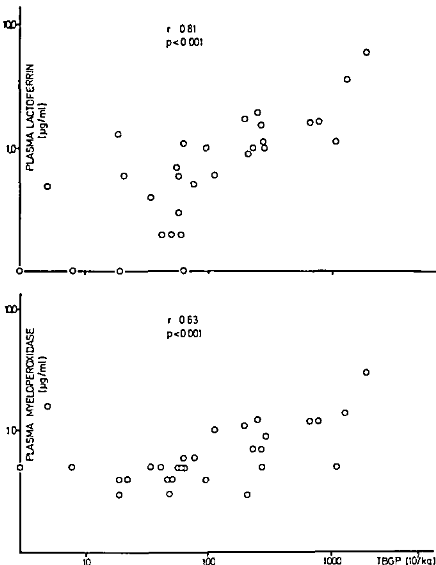


Fig 2 Relationship between the plasma concentration of lactoferrin and myeloperoxidase and the total number of neutrophils in the blood (*TBGP* = total blood granulocyte pool)

DISCUSSION

The present normal values for myeloperoxidase and lactoferrin differ from those published previously (13 14) The earlier results were obtained on serum samples rather than plasma and a less sensitive radioimmunodiffusion method was employed Although in those studies there were no indications of major in vitro leakage of leukocyte constituents results presented for lactoferrin by Rumke et al (19) do indicate that such leakage may occur and that EDTA plasma rather than serum should be used In the present studies as well comparative measurements showed that both proteins tended to occur in somewhat higher concentrations in serum than in EDTA plasma

Rümke et al (19) employing a double antibody radioimmunoassay technique reported a 99% range for plasma lactoferrin in normal women of 0 27–2 39 µg/ml whereas in the present study with a similar method the normal range for plasma lactoferrin was found to be 0 13–0 42 µg/ml No explanation can be offered for this difference

With regard to myeloperoxidase no assay data are available for comparison This protein for unknown reasons is difficult to radioiodinate without reduction in immunoreactivity as noted previously (23) Attempts to improve the method are in progress

This study has demonstrated a significant correlation between the neutrophil kinetic parameters

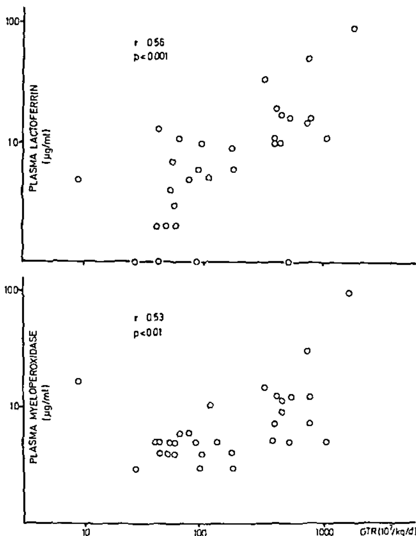


Fig 3 Relationship between the plasma concentration of lactoferrin and myeloperoxidase and the neutrophil turnover rate (*GTR* = granulocyte turnover rate)

TBGP and GTR and the plasma concentrations of myeloperoxidase and lactoferrin over a wide range of kinetic values and plasma protein concentrations This correlation is highly suggestive that neutrophilic granulocytes are the main source of these two proteins in the plasma as has been suggested (13 14)

Lactoferrin correlated better with the neutrophil kinetic parameters than did myeloperoxidase This is somewhat surprising since myeloperoxidase is known to be produced exclusively in myeloid cells (20) whereas lactoferrin presumably is produced in other tissues as well (16) The good correlation of lactoferrin with the TBGP must mean however that lactoferrin from sources other than neutrophilic granulocytes is of no major importance for the plasma content

After release from the bone marrow the neutrophilic granulocytes pass through the blood with a mean passage time of around 10 hours in normals Whereas a small proportion of the cells die in the blood stream from senescence (6) most neutrophils leave the circulation in a random fashion after leaving the blood stream some are believed to die in the tissues whereas an unknown number are lost through the bronchi and the alimentary tract If an intracellular protein is released from neutrophils within the circulation either after disintegration of the cells dying the the circulation or because it is released through intact cell membranes the plasma concentration of the protein would be expected to reflect the TBGP In contrast if the protein is released from disintegrating cells in the tissues and then diffuses back to the plasma the plasma con

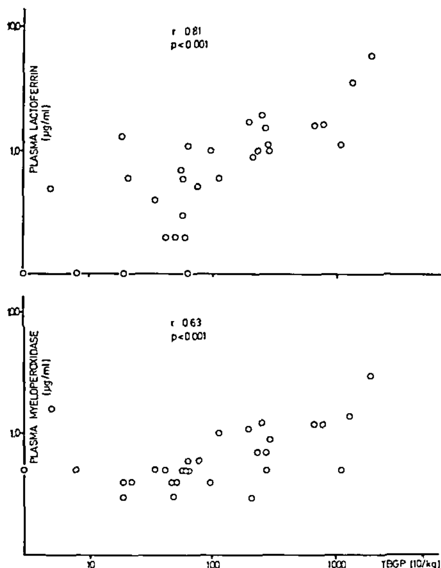


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Sudden Death in Rheumatoid Arthritis with Atlanto-axial Dislocation

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ABSTRACT A post mortem material of 11 consecutive cases of severe atlanto axial dislocation (a a d) with cord compression is reported The total number of deaths from rheumatoid arthritis (RA) during the period of 5 years was 104, and all were autopsied Neurological symptoms correlated poorly to fatal a a d Hemiplegia was found in three cases, one of which however, was caused by thrombosis cerebri Spastic signs were transiently recorded in two patients and dysphagia in a further two Five patients had a history of recent vomiting A a d was the sole or main cause of death in 8 cases and contributory in 2 Sudden death occurred in 7 of the cases Only 2 cases had obtained a correct diagnosis intra vitam The CNS findings at autopsy consisted of cord compression (11/11 cases), cord malacia (2/11) and cerebral oedema (3/11) One case had polyarteritis and renal amyloidosis and one pulmonary carcinoma with metastatic spread Signs of active inflammation in the axial joints were present in 4 cases This study based on systematic post mortem examinations, revealed an unexpectedly high and not previously reported incidence of fatal medulla compression in RA patients with a a d (10%)

Atlanto-axial dislocation (a a d) is a well known feature in rheumatoid arthritis (RA) occurring in not less than 25% of most hospital series (4 13) The natural history in the latter retrospective study seems to indicate that on the whole the afflicted patients do not have a higher mortality rate than RA cases without C 1–2 instability Furthermore spinal cord compression was only seen in 3 of 52 deaths

However one weakness of this and other studies lies in the lack of consistent post mortem examination with a suitable technique enabling the visualization of the cervical spinal tract We here report on 11 cases of autopsy proven cord compression found during a limited period of 5 years in a general hospital for chronic diseases indicating a greater ultimate importance of this condition than is generally appreciated Clinical warning signs were sought retrospectively from the records

MATERIAL AND METHODS

The cases emanated from a hospital for chronic disease serving a city population of approximately 250000 inhabitants The number of beds and of RA patients cared for each year are shown in Table I The autopsy rate was 90% and the autopsies were performed with a standardized technique mostly by one and the same pathologist The foramen occipitale region was examined in all cases regardless of clinical diagnosis Cases of minor translocation of the odontoid process without obvious cord compression were not included in this study In 8 of the 11 cases blocks were removed containing the upper cervical spine and an adjacent part of the skull base The specimens were fixed in formalin sawed sagitally decalcified and examined histologically The compressed medullas and brains were examined by routine histological methods

RESULTS

Table I shows the number of all autopsies in the hospital during 1969–74 to be around 500 each year The number of RA patients autopsied each year varied between 8 and 25 and was approximately 5% of the total mortality in the hospital All cases in this series had a severe form of disease and were in functional class III or IV on admission The Waaler Rose test was positive in 9 cases and not recorded in the remaining two The duration of the disease

Table I *Number of hospital beds RA cases cared for and autopsies performed*

Year	Beds	RA cases	Autopsies Total	RA cases	A a d cases
1969	872	46	560	19	1
1970	792	41	442	8	2
1971	746	40	462	25	1
1972	744	43	472	21	4
1973	744	34	501	20	1
1974*	725	50	406	11	2
Total			2 843	104	11

* Jan 1 – Oct 30

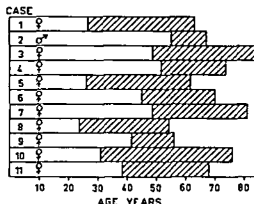


Fig 1 Sex distribution duration of disease and age at death in 11 cases of a a d

was 12–45 years (mean 29 8) (Fig 1) Ten of the patients were women The age at death varied between 54 and 81 years (mean 68 3) Hospitalization at death had lasted from one month to 26 years Six of the patients had been hospitalized for more than one year and 8 for more than 6 months Neck pain at rest and on motion was recorded as pronounced in 7 cases and in 4 of these (nos 2 4, 6 and 8) neck symptoms had been one of the presenting signs of RA

Neurological signs are summarized in Table II In 3 cases apparently no major neurological signs were recorded Case 2 was treated under the diagnosis of thrombosis cerebri but had atypical pains in the paralysed limbs The vertigo in case 3 occurred in connection with indomethacin therapy Case 7 had a number of episodes with clonic cramps and became disoriented a month prior to death Case 9 had 2 months before death an episode with vomiting followed by generalized clonic cramps but no Babinski s sign Case 10 suffered a humerus fracture 1 month prior to death and was mentally deranged from then on She subsequently developed a flaccid hemiplegia with a transient Babinski s sign Case 11 had for many months swallowing difficulties and it was also noted that her voice had changed and become hoarse

Standard X ray examination of the neck had been performed in 4 of the cases and although various degrees of erosion and sclerosis were found in no instance was a a d visible In cases 2 and 9 X ray examination including lateral views in forward flexion had been performed and both showed unequivocal subluxation the distance between dens and atlas being 15 and 8 mm respectively In case 10 X ray examination was requested shortly before death but could not be performed due to the patient s poor condition In the remaining 4 cases no X rays of the neck were taken

Terminal symptoms Table III summarizes the dominating symptoms immediately preceding death Seven of the patients died suddenly mostly

Table II *Neurological symptoms*

Case no		Duration before death
1	Not reported	
2	Hemiplegia with pain	4 mo
3	Vertigo suspicious extensor reflex	1 mo
4	Not reported	
5	Hemiplegia	1 mo
6	Not reported	
7	Disoriented convulsions	1 mo
8	Dizziness	4 mo
9	Seizures	2 mo
10	Transient hemiplegia dysphagia	1 mo
11	Dysphagia transient extensor reflex	14 mo

Table III *Terminal symptoms*

Case no	
1	Breathing difficulties sudden death
2	Weakness circulatory failure
3	Sudden death while washing her face
4	Respiratory arrest
5	Weakness fever circulatory failure
6	Abdominal pains chills vomiting
7	Sudden death vomiting
8	Sudden death vomiting
9	Hypotension slowly progressing coma
10	Progressive coma
11	Respiratory stridor sudden death while asleep

Table IV *Macroscopic and microscopic post mortem findings*

Case no	Gross anatomy: Medulla	Gross anatomy: Brain	Histology of axial joints	Other lesions
1	Compression	Oedema	Not examined	Renal concrements
2	Compression with malacia	Oedema	Not examined	Stomach ulcer
3	Compression	Normal	Active inflammation rheumatic granulomas	Pyloric ulcer polyarteritis amyloidosis
4	Compression	Normal	Non specific inflammation fibrosis	Ulcus scar in stomach
5	Compression	Malacia of parietal lobe (unrelated)	Non specific inflammation fibrosis	Stomach ulcer pancarditis
6	Compression	Congestion	Fibrosis	Acute peritonitis
7	Compression	Normal	Active inflammation rheumatic granulomas cancer metastases	Bronchial cancer with metastases
8	Compression	Congestion	Fibrosis	Rheumatic carditis acute stomach ulcer
9	Compression with malacia	Oedema	Not examined	Psoriasis
10	Compression	Congestion	Fibrosis odontoid exostoses	Stomach ulcer
11	Compression	Subtenorial oedema	Fibrosis odontoid exostoses	Rheumatic perimyocarditis

while resting in supine position Five patients had experienced nausea and vomiting during the last week of life but only one patient (no 6) actually vomited in agony In four patients death occurred after slowly progressing weakness

Post mortem findings Table IV summarizes the post mortem findings Common in all cases was displacement upwards and backwards of the odontoid process protruding into foramen magnum with impingement on the medulla (Fig 2) The protruded odontoid narrowed the spinal canal up to 4 mm sagitally (Fig 3) The lower part of the medulla was subsequently caught and pressed against the posterior wall of foramen occipitale and adjacent vertebrae The medulla was flattened and showed a compression furrow just below the olivae (Figs 4 and 5) In case 11 the displaced odontoid protruded 25 mm above the level of the foramen occipitale (Fig 6) and compressed the medulla at the pontine border In this case the membranes covering the odontoid were perforated

The compressed medullas seemed to be less vas

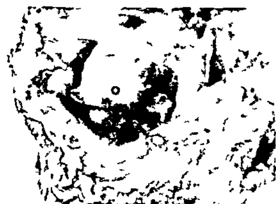


Fig 2 Foramen magnum from above O=apex of the odontoid M=flattened medulla pressed against the posterior edge of the foramen Case 8 Magnification ×1 3



Fig 3 Sagittal section of the occipito-cervical block O=odontoid pitted into foramen magnum A=anterior arch of atlas C=clivus Atlanto-axial joint obliterated by fibrous tissue Case 8 Scale 75%

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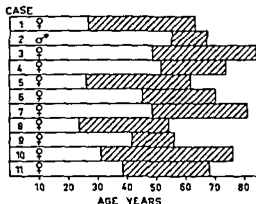


Fig 1 Sex distribution duration of disease and age at death in 11 cases of a a d

was 12–45 years (mean 29 8) (Fig 1) Ten of the patients were women The age at death varied between 54 and 81 years (mean 68 3) Hospitalization at death had lasted from one month to 26 years Six of the patients had been hospitalized for more than one year and 8 for more than 6 months Neck pain at rest and on motion was recorded as pronounced in 7 cases and in 4 of these (nos 2 4 6 and 8) neck symptoms had been one of the presenting signs of RA

Neurological signs are summarized in Table II In 3 cases apparently no major neurological signs were recorded Case 2 was treated under the diagnosis of thrombosis cerebri but had atypical pains in the paralysed limbs The vertigo in case 3 occurred in connection with indomethacin therapy Case 7 had a number of episodes with clonic cramps and became disoriented a month prior to death Case 9 had 2 months before death an episode with vomiting followed by generalized clonic cramps but no Babinski s sign Case 10 suffered a humerus fracture 1 month prior to death and was mentally deranged from then on She subsequently developed a flaccid hemiplegia with a transient Babinski s sign Case 11 had for many months swallowing difficulties and it was also noted that her voice had changed and become hoarse

Standard X ray examination of the neck had been performed in 4 of the cases and although various degrees of erosion and sclerosis were found in no instance was a a d visible In cases 2 and 9 X ray examination including lateral views in forward flexion had been performed and both showed unequivocal subluxation the distance between dens and atlas being 15 and 8 mm respectively In case 10 X ray examination was requested shortly before death but could not be performed due to the patient s poor condition In the remaining 4 cases no X rays of the neck were taken

Terminal symptoms Table III summarizes the dominating symptoms immediately preceding death Seven of the patients died suddenly mostly

Table II *Neurological symptoms*

Case no		Duration before death
1	Not reported	
2	Hemiplegia with pain	4 mo
3	Vertigo suspicious extensor reflex	1 mo
4	Not reported	
5	Hemiplegia	1 mo
6	Not reported	
7	Disoriented convulsions	1 mo
8	Dizziness	4 mo
9	Seizures	2 mo
10	Transient hemiplegia dysphagia	1 mo
11	Dysphagia transient extensor reflex	14 mo

Table III *Terminal symptoms*

Case no	
1	Breathing difficulties sudden death
2	Weakness circulatory failure
3	Sudden death while washing her face
4	Respiratory arrest
5	Weakness fever circulatory failure
6	Abdominal pains chills vomiting
7	Sudden death vomiting
8	Sudden death vomiting
9	Hypotension slowly progressing coma
10	Progressive coma
11	Respiratory stridor sudden death while asleep



Fig 8 Rheumatic granuloma in the obliterated atlanto-axial joint Note irregularly eroded contours of the adjacent cortical bone of the odontoid Case 7 Magnification ×97 5

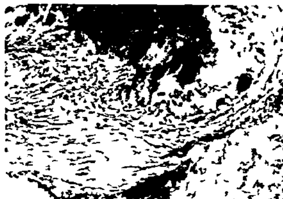


Fig 9 Rheumatic granuloma in Fig 8 magnified ×252

with tissues outside the bones The fibrous tissue in cases 4 and 10 was focally infiltrated by plasma cells and lymphocytes In cases 3 and 7 there were moreover specific rheumatic granulomas with fibrinoid necroses surrounded by palissaded histiocytic cells (Figs 8 and 9)

Causes of death Analysis of autopsy findings as related to clinical symptoms unequivocally implicate a a d with cord compression as the only cause of death in 5 cases (Table V) In the remaining cases other causes of death must also be considered Thus in case 5 a thrombotic occlusion of the medial cerebral artery with a corresponding malacia of the parietal lobe was the obvious cause of death Case 3 had a moderate uraemia with a stable serum creatinine of 4 mg/100 ml and renal amyloidosis was found to be the underlying cause The unexpected sudden death of the patient was however judged to be due to spinal cord compression In case 6 a gangrenous lesion in the distal part of ileum had perforated In case 7 neurologic symptoms together with sudden death led to the clinical suspicion of intracranial metastasizing carcinoma However the post mortem examination showed no CNS tumor growth and the immediate cause of death was considered to be the a a d In case 10 the necropsy showed an acute pyelonephritis and the infection was listed as the main cause of death and the cord compression as contributory

Table V *Causes of death*

Case no	Main cause of death	Accessory cause of death
1	a a d	
2	a a d	
3	a a.d	Renal amyloidosis
4	a a d	
5	Cerebral artery thrombosis	a a d ?
6	Peritonitis	a a d
7	a a d	Pulmonary adenocarcinoma
8	a a d	Rheumatic carditis
9	a a d	
10	Acute pyelonephritis	a a d
11	a a d	

DISCUSSION

The present series of fatal atlanto-axial dislocation stresses the diagnostic difficulties encountered when dealing with severely disabled patients with rheumatoid arthritis In only two of 11 cases was the dislocation diagnosed before death and in one more case it was suspected shortly prior to death On retrospective analysis of neurological symptoms and signs only non specific patterns could be found and three cases apparently had no neurological findings at all Spastic symptoms were only noted in three or four of the patients These results are in accord with published clinical reports of systematically examined hospital patients with RA (4) Thus neurological signs are not helpful for diagnosing patients with a a d or pointing out those at risk of developing fatal cord compression

Whereas the incidence of a a d is accurately known from earlier studies only indirect and unreliable data are reported regarding medullary compression caused by a a d These patients die often unexpectedly with a clinical picture compatible

with for instance such common conditions as cerebrovascular accidents myocardial infarction or pulmonary embolism Autopsy rates may be low and even when autopsy is performed one may miss the condition if skull opening and removal of the brain is performed without close observation of the foramen magnum and upper cervical region Consequently autopsy reports on spontaneous fatal cord compression in RA are very few and consist of 6 cases (1 5 7 8 14 16) In one of these cases both vertebral arteries were thrombosed In addition Hauge (6) described two deaths occurring after attempted surgical decompression Discussion of a a d not related to RA is beyond the scope of this paper for references see Davis and Markely (5) and Wadia (15)

Many RA patients die at home or in asylum and post mortem examinations are seldom performed This situation is illustrated by Smith et al (13) describing a group of 130 patients with clinically observed rheum cervical luxation In only one of 46 patients dying during the follow up period was there a clinical suggestion of fatal cord compression however in no case was a post mortem examination performed

These authors conclude that the luxation per se in the absence of cord or brainstem involvement does not shorten survival However one must question the validity of their statement on the ground that post mortem studies on control and luxation cases are incomplete Fatal cord compression deaths could be hidden in the both groups The study of Mathews (10) also suffers from the lack of autopsy data on the deceased patients

Werne (17) established the dependence of atlanto-axial stability upon intact transverse and alar ligaments and Ball and Sharp (1) also stressed the importance of osteoporotic changes and erosions caused by granulation tissue in the weakening of these ligaments Our specimens were sawn sagitally in order to visualize odontoid protrusion This method precluded gross examination of the ligaments which in the histological sections were scarcely discernible in the fibrous tissues surrounding the odontoid One histological finding which may be of significance is that of rheumatoid granulomas in two of our cases as well as in two of those published by others (5 8) indicating a rather malignant type of RA

Fatal impingement on the medulla seems to be a final event in a slowly progressing displacement of the odontoid process The death can be precipitated by sudden head movements such as induced by vomiting However an eliciting factor could not always be identified As only minimal lesions could be observed in the compressed medullas the death is apparently due to sudden ischemia of the reticular substance containing respiratory and circulatory centra This supposition is corroborated by cases 2 and 9 developing malacia of the medulla with death coming less suddenly We conclude that a a d occurs in approximately 10% of RA patients dying in a Swedish nursing home population Assuming a total incidence of roentgenologically detectable a a d of 30% among these patients the mortality rate would be around 25%

In view of the encouraging results with surgical fusion reported recently by Brattstrom and Granholm (2 3) one must consider this therapeutic possibility even in cases without cord or brain stem symptoms especially since the majority of the patients suffer from pains in the neck region Cervical collars are often poorly tolerated and ineffective as stabilizers

The role played by corticosteroid treatment in the development of a a d is controversial Smith et al (13) found an adverse effect on the progression Among our patients 3 had received no steroids and 2 had steroids only for brief periods and in low doses These observations do not support a major importance of steroid treatment for the development of fatal a a d

As pointed out by Werne (18) Robinson (12) and Ornilla et al (11) a a d has to be considered as a possible risk in connection with anesthesia for surgery in RA Physiotherapeutic manipulations may be dangerous too

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Skeletal Status in Patients with Rheumatoid Arthritis

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ABSTRACT The total body calcium – estimated from the bone mineral content in the distal part of the forearm, measured by means of photon absorptiometry – has been determined in 15 patients with rheumatoid arthritis None of the patients were treated with steroids and all were relatively mobile The results indicate that osteoporosis is uncommon in such patients

Recent investigations have postulated that osteoporosis estimated from X ray pictures is a common phenomenon in patients with rheumatoid arthritis (1 8 9) The etiology of bone loss is not understood but an explanation is often sought in various multiple factors such as immobilization treatment with corticosteroids or other anti inflammatory agents However the specific role of each factor is poorly defined and it is still not clear whether rheumatoid arthritis in itself leads to osteoporosis

We have investigated the bone mineral content (BMC) in a group of relatively mobile patients with rheumatoid arthritis (without corticosteroid treatment) in order to investigate whether and to what degree rheumatoid arthritis in itself leads to osteopenia We studied this by measuring the BMC in the distal part of the forearm by photon absorptiometry This parameter is well correlated to total bone calcium (3 4 5 6)

MATERIAL AND METHODS

Eleven women and four men aged 26–59 years (mean 46) consented to the study All patients had rheumatoid arthritis defined according to the American Rheumatism Association criteria Mean duration of the illness was 9 5 years (range <1–42) Nobody was being or had been treated with corticosteroids and all were receiving one or two anti inflammatory agents such as acetyl salicylate or phenylbutazone They were all relatively mobile patients

BMC was determined by direct photon absorptiometry on both forearms (Fig 1) Antebrachium is fixed in a plexiglass container filled with distilled water Water is used since it has nearly the same coefficient of absorption as plexiglass and soft tissue The source of radiation (25 mCi ^{125}I) and the detector are fixed in holders on either side of the bones to be examined A mechanical scanner displaces the detector and the source perpendicular to the longitudinal axis of the bones The transmission of photons through the bones depends on the BMC Cameron et al (2) showed a direct relation between the absorption of photons from ^{125}I and BMC In our modified version of the method (3) the BMC is expressed in arbitrary units as a mean value of six scans from each forearm The coefficient of variation of duplicate measurements in the same patient on different days is of the order 3–4% BMC of the forearm is fairly well correlated to the body s total calcium content (3 4 5 6) The BMC value in each patient was compared with the values in a group of 127 normal subjects matched for age and sex (4) in such a way that the patient s value was classified using the mean and S D in the corresponding age and sex dependent subgroup Furthermore the patient s BMC value was expressed as a percentage of the corresponding normal mean

RESULTS

The percental value of BMC in the patients did not correlate significantly to the duration of illness and the patients were accordingly considered as a sample of the population of relatively mobile rheumatoid arthritis patients without corticosteroid treatment

The BMC values in the patients scattered around the corresponding normal mean and no patient had a BMC value lower than the normal range (mean ±2 S D) The group mean for the 15 patients amounted to 94% (S D 18%) of the corresponding normal mean This is not significantly different from the normal mean (100% S D 16%) according to either the *t* test for differences between averages or Wilcoxon s test

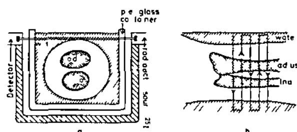


Fig 1 Principle for the measurement of bone mineral content by direct photon absorptiometry on the forearm (*a*) Section through system showing plexiglass container and U shaped holder with radioactive source and detector (*b*) System seen from volar surface showing scanning movements of source and detector

DISCUSSION

The results indicate in spite of the relatively few patients that osteoporosis in rheumatoid arthritis is unusual in relatively mobile patients treated with ordinary anti inflammatory drugs Mueller and Jurist (7) found in six patients treated with corticosteroids that the BMC (investigated with almost the same technique radius alone being studied with a non scanning procedure) was significantly decreased to nearly 70% of the value in six matched controls without steroid treatment Furthermore they found that the mean BMC in 20 patients with rheumatoid arthritis without steroids did not differ significantly from the mean in 20 matched controls The patients BMC amounted to 95% of the corresponding normal mean The present study confirms these findings our mean value amounting to 94% of normal

From these two recent studies it is tempting to conclude that rheumatoid arthritis *in itself* does not lead to significant osteoporosis However further studies are clearly necessary to test whether and to what degree osteoporosis is found in this disease A question of particular interest is the part played by immobilization in the development of osteoporosis in rheumatoid arthritis

ACKNOWLEDGEMENT

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High Pacemaker Implantation Rate Following "Cardiogenic Neurology"

Results in 129 Consecutively Paced Patients

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ABSTRACT This paper gives data on symptomatic patients with bradycardia found not at random but as the result of greatly expanded facilities for recognition of arrhythmias The incidence of patients with symptomatic bradycardia was very high Most patients had intermittent arrhythmias and diagnosis was often only possible after long term ECG screening Symptoms were as severe and relief of symptoms with treatment was as good, as in pacemaker treated patients described by others Our conclusion is that indications for arrhythmia screening should be increased as the diagnosis and treatment are very worthwhile We refer to the systematized active diagnosis and treatment of arrhythmias in patients with cerebral symptoms as cardiogenic neurology

In the Uddevalla region in Sweden facilities for recognition of arrhythmias have been greatly expanded in the recent few years The region supports a population of about 120000 It is served by one 800 bed hospital and two county hospitals with about 60 beds each There are adequate facilities for geriatric and long term psychiatric care There are about 50 general practitioners most of them employed on a full time basis by the state or community

ARRHYTHMIA DETECTION FACILITIES AND POLICY

In 1968 a coronary care unit (CCU) with four beds was opened in the main hospital Two years later we began to monitor patients with minor cerebral ischemic episodes who would formerly have been considered to have cerebrovascular or neurological disease A concept of cardiogenic neurology that is systematized active diagnosis and treatment of cardiac arrhythmias in patients with cerebral symptoms was formulated and published at both local and general medical meetings (2 3 4)

In 1971 another four beds with monitoring facilities were created in the main hospital together with one bed in each county hospital thus one bed per 12000 inhabitants At the same time nurses in the area received detailed instructions in ECG recording techniques and in the diagnosis and treatment of cardiac arrhythmias

Our CCU now also serves as a stroke monitoring unit (24 31) We however especially admit patients suggestive of transient general ischemic attacks This has yielded an arrhythmia detection rate similar to that of Walter et al (37)

As the results of the arrhythmia detection program became apparent they were conveyed to the general practitioners This resulted in an improved awareness of patients with symptomatic bradyarrhythmias whether of the persistent or intermittent type

PATIENTS

Up to the end of 1972 129 patients in the area had permanent pacemakers implanted due to symptomatic bradycardia This paper is based on follow-up data obtained at the end of 1973 Forty-one patients were below and 88 above the age of 70 The age distribution is given in Table I

The highest arrhythmia detection rate was found in 1972 with 42 new pacemakers inserted This figure corresponds to 350/1 mill people The mean for all Sweden during that year was 123/1 mill Of these 42 patients 21 were men and 21 women 35 were aged 70 or more (In 1973 there were 53 implantations of permanent pacemakers)

Symptoms and arrhythmias

Sinus bradycardia or a severe sinus arrhythmia was seen in four patients All of them had Stokes Adams attacks Sinoatrial block was encountered in 11 patients Nine of them had Stokes Adams attacks and two had periods of dizziness Sick sinus syndrome as described by Ferrer (13 14) and always complicated by tachycardia was present in seven patients Five of them had syncopal attacks

Table I *Patients with permanent pacemakers in the Uddevalla region up to 1972*

Age at implantation (y)	N
<49	4
50-59	7
60-69	30
70-79	51
80-89	34
>90	3
Total	129

and one had psychotic episodes The seventh during an acute myocardial infarction (AMI) developed an arrhythmia of such persistence that it was considered inevitable that symptoms would arise

Atrial fibrillation with normal rate but with asystoles (R R interval >2.5 sec) or intermittent severe bradycardia was seen in nine patients Eight had Stokes-Adams attacks The ninth developed a persistent arrhythmia after infarction and was confused during periods of asystole Atrial fibrillation with slow ventricular rate was seen in seven patients Three of them had Stokes-Adams attacks and three had attacks of dizziness The seventh received a pacemaker so that digitalis treatment could be instituted Slow nodal rhythm was seen in five patients all of whom had Stokes-Adams attacks

Intermittent second degree atrioventricular block was seen in four patients Three had Stokes-Adams attacks and the fourth had persistent intermittent arrhythmia following a myocardial infarction. Intermittent third degree atrioventricular block was found in 26 patients 18 of whom had Stokes Adams attacks Two patients had dizziness and one suffered from confusion during the bradycardia. One patient, whose arrhythmia was discovered during a routine health survey had a pacemaker implanted even though he was symptom free Four patients had persistent intermittent arrhythmias following myocardial infarction.

Chronic second degree atrioventricular block was seen in two patients Both received pacemakers prior to digitalis treatment Chronic third degree heart block was encountered in 54 patients Thirty nine had Stokes Adams attacks and three had dizziness Of the remaining 12 patients seven had this arrhythmia in association with AMI Three had heart failure one abdominal angina and one was symptom-free

The arrhythmias and the number of patients with Stokes-Adams attacks in each group are summarized in Table II

If patients had been on digitalis at the time when arrhythmia was recorded the drug was discontinued Plasma digitalis concentrations were determined in most of those instances Up to several weeks were allowed before the final decision was made of therapy When arrhythmias were seen on the monitoring screen in the CCU they were recorded and analyzed in detail All arrhythmias were documented by recordings

CLINICAL FEATURES

In 35 patients the bradycardia was first noticed while patients were being monitored in the CCU for suspected or proven myocardial infarction The other cases were monitored according to cardiogenic neurology or because of a history of bradycardia

Fourteen patients received permanent pacemakers for persistent bradycardia following an AMI even though there was no evidence of Stokes Adams attacks or any other major symptoms Patients with AMI received pacemakers only when there were persistent slow rhythms or when the clinical situation became critical with bradyarrhythmias complicated by ventricular tachycardia or fibrillation

Transvenous pacing with an external unit was used initially Only patients who needed permanent pacing are included in this paper (16 22 34 38) Two patients had paroxysmal ventricular tachycardia and bradyarrhythmia They needed pacing at a high rate (5 8 35) Fourteen patients received pacemakers to make digitalization possible One of them had an AMI and another died of AMI two months after pacemaker implantation The remaining patients in this group are alive with observation times of up to three years

In the last year several patients who presented with a history of confusion were found to have bradyarrhythmias when examined One patient who was detained in a mental hospital with senile psychosis returned home after her bradyarrhythmias had been diagnosed and treated

Table II *Arrhythmias and Stokes Adams attacks in the 129 Uddevalla patients*

Arrhythmias		Stokes Adams attacks
Sinus bradycardia/arrhythmia	4	4
Sinoatrial block	11	9
Sick sinus syndrome	7	5
Atrial fibrillation with asystoles	9	8
Slow atrial fibrillation	7	3
Slow nodal rhythm	5	5
A V block		
Intermittent 2nd degree	4	3
Intermittent 3rd degree	26	18
Persistent 2nd degree	2	0
Persistent 3rd degree	54	39
Total	129	86

Table III *Symptoms and signs of cardiac disease on admission in 75 patients with intermittent bradyarrhythmia*

Symptom or sign	No of pats
Chest pain 15 Heart failure 18 Spontaneously mentioned slow pulse 1 Bradycardia requiring pacing 2	26
None of the above	49
History of slow pulse obtainable	12

The records of 38 patients contained a history of medical care or hospital treatment for symptoms or diseases which in retrospect could have been due to cardiac arrhythmias Most common were vertigo ($n=9$) syncope ($n=7$) cerebrovascular thrombosis ($n=7$) epilepsy ($n=7$) and Méniere s disease ($n=3$) In addition one case each of Addisonian crisis repeated urinary retention paroxysmal blindness alcohol intoxication and senile psychosis were recorded

The records of 17 patients contained a history of traumatic injuries that needed hospital treatment some time before the arrhythmia was actually diagnosed Most common were cerebral concussion and/or fractures ($n=12$) One patient in this category had had burns and the remaining four other injuries

There were 75 patients with intermittent bradycardia Forty nine of them would not have been considered as cardiac on admission At initial examination only two showed a bradycardia which needed closer observation for possible pacemaker treatment Details are given in Table III

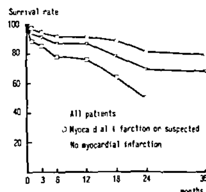


Fig 1 Survival rates for patients who received pacemakers in relation to possible myocardial infarction or without relation to myocardial infarction

Table IV *Twenty three deaths among 129 permanently paced patients*

Pat no	Cause of death	Interval between implantation and death (mo)
1[a]	AMI pump failure	0–1
2	AMI rupture of left ventricle	0–1
3	AMI dislocation	0–1
4[a]	AMI ventricular fibrillation	0–1
5	AMI	0–1
6[a]	AMI ventricular fibrillation dislocation	0–1
7	Thrombotic thrombocytopenic purpura	0–1
8	Cerebral thrombosis	0–1
9	Reinfarction	2
10[a]	Reinfarction	2
11	AMI	2
12	Cerebral thrombosis	2
13	Cerebral thrombosis	4
14	Heart failure	5
15	Reinfarction	5
16	Heart failure	14
17	Heart failure	16
18[a]	Sudden death exit block?	17
19	Mesenteric thrombosis	17
20	Uremia exit block?	17
21	Cerebral thrombosis pulmonary embolism	21
22	Uremia	22
23	Sudden death exit block?	37

[a] The patient received pacemaker on account of an arrhythmia caused by infarction

Mortality

There was a marked difference in mortality according to the presence or absence of myocardial infarction

The group of 35 patients whose bradycardia was diagnosed while being monitored for a definite or suspected myocardial infarction had a 1 year survival of 76% and the 2 year survival was 50% Among the other 94 patients the corresponding figures were 94% and 81% respectively Fig 1 presents the survival rates for both groups

The intervals between pacemaker implantation and death are given in Table IV Six of eight patients who died within a month had an AMI Eleven deaths were caused by cardiac disease including two cases of ventricular fibrillation and infarction Two deaths were attributed to electrode dislo-

Table V *Social situation before and after pacemaker treatment for 94 patients with bradycardia not related to myocardial infarction*

	Before	After
Own home	85	74
Home for the aged	8	11
Long term medical care	1	7
Death		2

cation This happened in patients with previous myocardial infarction Three late deaths may be attributable to long term pacing complications Five cases of thrombotic disease were lethal Four of them were cerebral and one mesenteric Finally uraemia and thrombotic thrombocytopenic purpura accounted for one death each There were no deaths from malignancy

Social situation before and after pacing

In 1972 38 of the patients were living at home when the bradyarrhythmia was diagnosed Following treatment 35 of them returned home Two went to homes for the aged and one to a geriatric ward

In all there were 94 patients whose bradyarrhythmia was not related to myocardial infarction The social circumstances for these 94 patients before and after treatment are given in Table V

Pacemakers

Early pacemakers were implanted at Karolinska Hospital in Stockholm Later we were assisted by the Cardio thoracic Department of Sahlgren s Hospital in Gothenburg Since the beginning of 1972 all the pacemakers have been implanted by surgeons of our hospital (2)

During the first years some patients with persistent bradycardias received fixed rate pacemakers (70 Elema 4 Medtronic) but in recent years we have only used the on-demand variety (75 Elema 22 Medtronic) We have changed a number of fixed rate pacemakers to demand units after seeing that some patients with persistent third degree heart block returned with sinus rhythm and interference Other investigators have noted a similar development (29) Eight patients with evidently good atrial activity in combination with persistent complete heart block received atrial triggered pacemakers (Elema)

Roughly a fourth of the patients had an early electrode dislocation No serious complications occurred in 1972 Wound infection and pacemaker pocket hematoma occurred in one case each Myocardial perforation valvular damage sepsis or endocarditis have not been found or suspected Anticoagulants were not used and there was no evidence either of thrombosis around the electrode or of embolic complications

DISCUSSION

Pacemaker implantation rates

At the Fourth International Symposium on Cardiac Pacing in Groningen the Netherlands 1973 reports were presented on pacemaker implantation rates in different countries in the industrialized world Implantation rates ranged from 6/1 mill in Japan to 123/1 mill in Sweden (19 23)

For several years the Uddevalla region has had a considerably higher implantation rate than other parts of Sweden In 1972 when the mean rate for Sweden was 123/1 mill we implanted 42 units corresponding to 350/1 mill When we first recognized this high ratio we were very concerned Had our indications been too wide or did there really exist such a large previously unrecognized group of patients with symptomatic bradycardia?

Interest in diagnosis of arrhythmia and facilities for monitoring

As our interest in symptomatic bradycardia increased facilities were expanded all over the area The number of monitoring beds was doubled and beds were created in the two small hospitals This

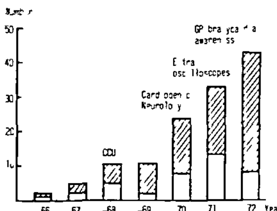


Fig 2 Pacemaker implantation rate in Uddevalla in relation to time and other factors ▨ – patients above 70 years of age

made it possible to monitor patients on much wider indications than before District and hospital nurses were invited to courses The district medical centers were furnished with full ECG facilities As results became available they were discussed at local medical meetings Fig 2 emphasizes how the increase in facilities and awareness resulted in an increased detection rate and an increase in the number of implanted pacemakers

Indications for pacing

Our indications for pacing seem to have been those generally accepted (6 10 11 18 27 28 29 36) and have not changed over the years The mortality in complete heart block is well established (21) as is the associated disability (12)

Apart from the fact that even moderate brady cardia may be dangerous (17) the more advanced forms often lead to considerable morbidity We have like others paced our patients with atrial bradycardias and they have had the same relief of symptoms as those with complete heart block (9 20 26 30 32 33)

It has been suggested that an arrhythmia itself could be an indication for treatment We have con sidered the arrhythmia in relation to associated symptoms before undertaking treatment Medical treatment was often tried but the results were dis appointing as seen in the monitoring Most patients with symptomatic bradycardia received a pace maker sooner or later

Clinical presentation

Many of the patients had had some accident in the years preceding the diagnosis of cardiac dys rhythmia and many had sought medical advice for symptoms that could in retrospect be related to an arrhythmia Edhag (12) found a similar accident pattern and similar symptoms that were possibly misinterpreted at the time This agrees with a report by McAllen and Marshall (25) and emphasizes that there is a need for better arrhythmia awareness

We conclude that in the general population there exists a larger group of patients with symptomatic bradycardia than was previously known

Only one of the 75 patients with intermittent bradycardia could himself relate symptoms to a slow pulse In only 13 cases could either the patient or a relative recall a slow pulse when directly questioned Only 26 of these 75 patients had some cardiac symptom or sign at first examination

Even though there may be no definite signs or symptoms of cardiac disease at initial examination one cannot exclude the possibility of an intermittent bradycardia Therefore one has to be aware of it and if a cardiac arrhythmia is suspected the patient must undergo long continuous monitoring before this possibility can be excluded with reasonable safety

The non cardiac appearance may be the reason why so many patients with serious bradyarrhyth mias can be found in hospitals for patients with senile dementia (1) It seems important to find these patients as soon as symptoms appear The cost of monitoring facilities is negligible in comparison with the long term geriatric care which might be required for many of these patients

Mortality

Most patients have died from cardiac disease thrombotic disease or from definite or suspected pacing difficulties The high number of patients dy ing from cerebral thrombosis is similar to data published by others (10 29) There were two pa tients with carcinoma after implantation One had an adenocarcinoma of the colon successfully re moved with no signs of metastases after three years of observation The other had a rectal carcinoma but refused surgical therapy One patient was known to have a well controlled cancer in the prostate gland when pacing was instituted

Indications for monitoring

We had gained a basic monitoring knowledge from experience in the CCU The Uddevalla unit was the second to be established in this country (7) It is a well known fact that some patients with myocardial infarction develop cerebrovascular symptoms On this basis we became interested in patients with acute cerebrovascular accidents We began to monitor these patients to see if some of them would prove to have silent myocardial infarctions needing antiarrhythmic or other cardiac treatment How ever we did not find many patients with myocardial infarction but our studies revealed that a surprising number of these patients were suffering from seri ous bradyarrhythmias Obviously it was futile to treat those who had suffered irreversible cerebral damage so we concentrated on those with minor or transient symptoms

The results of monitoring were encouraging out a third of this group proved to have a serious

bradyarrhythmia amenable to standard medical treatment In many cases this was done simply by adjusting medication others needed a pacemaker

Cardiogenic neurology

Neurological symptoms due to defective cerebral perfusion associated with cardiac arrhythmias are well recognized as a clinical problem The extent to which they occur their diagnosis and the practical value of successful treatment have however not been fully emphasized A concept of cardiogenic neurology was therefore evolved and has proved very valuable as a brand name for activities for these patients

We consider cardiogenic neurology to be the active diagnosis and treatment of arrhythmias in patients with cerebral symptoms Symptoms may be those of a general cerebral ischemic attack manifesting as syncope sudden dizziness confusion epilepsy or disturbed vision The arrhythmia may be a tachycardia or a bradycardia Treatment may be a pacemaker or simply a change of medication

CONCLUSIONS

Many more patients with symptomatic bradycardias were found in our area when interest was directed towards arrhythmia detection and the necessary facilities were expanded Therapy was very worthwhile and may for many patients have been the alternative to severe disability and long term medical care for a supposed cerebrovascular disorder It may be fruitful to expand arrhythmia detection facilities elsewhere and to make it a clinical challenge to find these patients

All elderly patients with cerebral symptoms which are not easily explained after a routine neurological examination should be thoroughly monitored to exclude an arrhythmia

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Routine Exercise ECG Three Weeks after Acute Myocardial Infarction

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ABSTRACT In the third week after acute myocardial infarction, mean 18 days exercise tests have been performed in 209 patients prior to discharge from the Coronary Care Unit The exercise was done on a bicycle ergometer with electrically controlled braking, starting at the load 300 kpm/min (equal to 50 W), increasing with 300 kpm/min every 6th min, aiming at a maximal symptom limited performance ECG, in 3 extremity leads and 3 precordial leads, and heart rate (HR) were continuously recorded, and blood pressure (BP) was measured every minute The most common cause for discontinuing exercise was fatigue (in 58 %) Anginal pain or dyspnoea was the cause in 23 8 % Only in 9 1 % was the exercise interrupted by the investigator because of rhythm disturbances or pronounced ST T changes Maximal work varied from 1 min exercise at 300 kpm/min to 6 min at 900 kpm/min (150 W) 18 % of all patients were able to work for 6 min at 600 kpm/min (100 W) HR increased on an average from 80 beats/min at rest to 129 beats/min at maximal work load Systolic blood pressure (SBP) increased on an average from 126 to 170 mmHg The maximal values reached during exercise were HR 170/min, and SBP 270 mmHg The product HR×SBP increased a little more than two-fold on an average ST T changes indicating myocardial ischaemia during exercise were observed in 70 % During exercise ventricular ectopic beats occurred in 42 % All rhythm disturbances provoked by exercise disappeared spontaneously shortly after work Persistent ECG changes reinfarction or other serious complications were not observed in connection with the exercise test It is concluded that an exercise test under controlled circumstances is safe in patients of all ages in the third week after myocardial infarction It is an objective measure of physical work capacity and describes the reaction to physical activity It gives a basis for advising return to normal life and is of great psychological importance to the patient

Strong physical activity has generally been deprecated in patients with acute myocardial infarction (AMI) as the myocardium is not expected to be completely healed until 8 weeks after such an event Therefore the myocardium should probably not be subjected to major increases in heart rate (HR) and blood pressure (BP) The potential hazards of exertion in these patients are considered to be precipitation of cardiac rupture formation of aneurysm congestive heart failure and serious disturbances of cardiac rhythm (6) Recently however many clinicians have begun to doubt the existence of these risks and recommend a gradual mobilization during the early phases of AMI and supervised physical training (6 12) In our department even complete mobilization from the first day without restrictions has come into general practice (11)

Consequently submitting the patients with AMI to a standardized exercise test before discharge from hospital seems justified The aim of the present study was

1) *To define the possible risk run by the patient* during physical performance as it is considered preferable that any adverse reaction to physical activity be observed under controlled circumstances

2) To describe the patient s reaction to physical exercise and his work capacity at the time of discharge and the limiting factors thus producing a safer basis for advising the patient regarding his return to work and to normal life

3) To demonstrate to the patient as well as to his relatives and employers that his capacity for physical performance is generally not severely affected by his past infarction

Table I *Reasons for non performance of exercise testing three weeks after myocardial infarction*

	Men	Women	Total
Cardiac incompensation	3	3	6
Anginal pain	1	2	3
Cardiac dysrhythmias	4	6	10
Locomotor and joint disorders	10	10	20
Age and/or bad general condition	6	6	12
Other incl pat s refusal	7	2	9
Total	31	29	60

Table II *Sex and age of patients performing and not performing exercise test three weeks after myocardial infarction*

	Performing exercise test	Not performing exercise test
Total no of pats	209	60
Men		
n	154	31
Age (y)		
Mean±S D	59±9	63±13
Range	33–83	36–89
Women		
n	55	29
Age (y)		
Mean±S D	62±11	71±11
Range	38–81	52–93

MATERIAL

From the beginning of 1972 exercise tests have been applied as a routine for all patients with AMI prior to discharge from the Coronary Care Unit (CCU) i e within the first three weeks after the onset of the infarction The present study comprises our material in the period Aug 1972 – May 1973 During these ten months 269 patients were discharged from the unit after AMI Further details regarding diagnostic criteria complications and treatment in the CCU are given elsewhere (11) The main lines were as follows

The criteria for the diagnosis of AMI were the finding of at least two positive parameters out of the following three typical symptoms typical ECG changes and typical changes in serum enzymes (GOT LDH LDH isoenzymes and CPK)

When the diagnosis of AMI had been established the patient remained in the CCU for a minimum of 21 days As to the regime and nursing care no restrictions were imposed From the day of admission the patients were allowed to be out of bed and walk around limited only by the 6 meter long ECG cable unless their general condition made this impossible After an average of 5–6 days according to individual need and the number of newly admitted patients they were moved to telemetric ECG monitoring and allowed to walk around all over the department

Exercise tests were performed in 209 (78%) of the 269 patients The reasons why 60 patients did not perform the tests are summarized in Table I It appears that cardiac symptoms—dysrhythmias anginal pain or incompensation—were the criteria in only 19 (32%) of the 60 cases that is in only 7% of all the patients discharged after AMI

Table II shows the distribution by sex and age of the patients performing and those not performing exercise test

At the time of the test 88 (42%) of the 209 patients were being treated with one or more of the following drugs digoxin (39 pats) diuretics (61 pats) β blockers (7 pats) or other antiarrhythmic agents (6 pats) or antihypertensive drugs (15 pats)

METHOD

The exercise test was performed on an average 18 days (S D =2 range 15–31) after the supposed onset of the AMI All patients were informed of the purpose of the investigation and only a few refused to participate (Table I)

At the beginning of the test the patient rested supine for at least 10 min while self adhesive ECG electrodes were placed in the deltoid regions in the groins and on the chest positions of V_1–V_4–V_6 and a BP cuff was placed round the upper arm Then the continuous registration of HR and ECG in 3 extremity leads and 3 precordial leads commenced and regular measurements of the BP every minute All measurements were continued through the following periods supine at rest for 3 min – sitting on bicycle at rest for 3 min – sitting on bicycle during exercise – supine at rest for at least 10 min

The ECG curves were recorded on a continuous paper strip by a 3–6 channel ECG apparatus (Mingograph 81 Siemens Elema) supplied with a tachometer From this the HR was taken down on a slowly moving penwriter (Servogor) and the BP measurements were added to this curve as shown in Fig 1

The graded exercise was done on a bicycle ergometer with electronically controlled braking (Siemens Elema A M 368) The load was increased stepwise from 300–600–900 kpm/min (equal to 50–100–150 W) staying on each step for 6 min in order to ensure a steady state The exercise was continued until the appearance of one of the following symptoms or signs anginal pain invincible fatigue or dyspnoea leg pain pronounced and rapidly increasing changes in the ST T segment ectopic beats of high and increasing frequency especially when multifocal and appearing in series and close to preceding T wave ectopic tachycardia prolongation of P–R interval or increasing width of QRS With these limitations the aim was a maximal symptom limited performance and the patient was encouraged to proceed until the appearance of one of the limiting factors No fixed limit to HR during exercise was applied as a reason for discontinuing the test

Statistical calculations were made by means of a *t* test for comparison of unpaired data

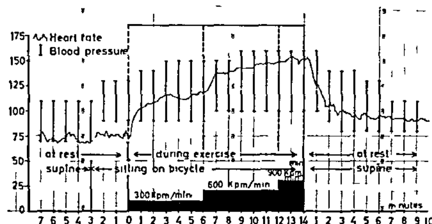


Fig 1 Heart rate blood pressure and work load during exercise test 17 days after myocardial infarction in a 58 year-old male patient

RESULTS

The reasons for discontinuing exercise are listed in Table III In only 19 cases (9 1%) was the exercise interrupted by the investigator because of the appearance of rhythm disturbances (frequent extrasystoles in 7 supraventricular tachycardia in 2 and ventricular tachycardia in 1) or of severe ECG changes (very pronounced ST T deviations in 6 and bundle branch block in 3) In all cases the rhythm disturbance or the ECG change disappeared within the test period More severe arrhythmias persistent ECG changes reinfarctions or cardiac rupture were not observed in connection with or shortly after the exercise test By far the most common cause for discontinuing exercise was plain insuperable fatigue (in 58 0%) Less frequent were cardiac symptoms such as anginal pain or an embarassing degree of dyspnoea (in 23 8%) The rest of the patients (9 1%) stopped exercise because of leg pain or poor cooperation

The total performance varied from 1 min exercise at 300 kpm/min to 18 min exercise at the stepwise increasing load 300+600+900 kpm/min each for 6 min As appears from Fig 2 75% were able to work for 4 min at 300 kpm/min 50% for more than 7 min at 300 and 600 kpm/min and 25% for more than 10 min at these loads

Table IV describes the findings regarding HR and SBP at rest and supine at rest and sitting on the bicycle and during maximal work load Comparing the figures at rest in supine and sitting positions there is a small significant increase in HR ($t=3$ 708 $p<0$ 001) but no significant difference between the values for SBP During maximal exercise the S D for all parameters was greater than at rest The wide range shows that some patients continued exercise until they reached rather high values of both HR (up to 170) and SBP (up to 270 mmHg)

As will be seen from Table V higher levels of work load were accompanied by higher values of maximal HR and SBP

In Table VI the maximal HR during exercise is related to the age of the patient expressed in four age groups As expected the maximal HR was considerably higher in the younger age groups the difference being statistically significant between each of the four groups There was a significant negative correlation between age and maximal HR ($r=-0$ 46 $p<0$ 001) The maximal work load expressed as the duration of the exercise in minutes was significantly higher in the younger age groups

The product of HR and SBP is presented in Table VII This product increased a little more than two fold during exercise in the whole group The maximal value varied with the reasons for stopping exercise as defined in Table III As shown in the lower half of Table VII the patients who stopped exercise because of general fatigue reached a

Table III *Reasons for stopping the exercise test performed three weeks after myocardial infarction*

	N	%
Arrhythmias	10	4 8
ECG changes	9	4 3
Angina pectoris	24	11 2
Dyspnoea	26	12 6
Fatigue	121	58 0
Other	19	9 1
Total	209	100 0

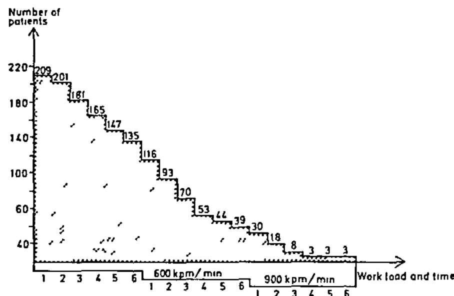


Fig 2 Work capacity in 209 patients subjected to exercise test three weeks after myocardial infarction (work loads equal to 50–100–150 W)

significantly higher value than did those who were stopped because of anginal pain and dyspnoea or because of cardiac arrhythmias and ECG changes

Table VIII shows the number of patients who developed ST T deviations during exercise estimated to indicate myocardial ischaemia Our criteria for this estimation were the finding of deviation of ST T segments in one or more ECG leads in the form of a horizontal or descending ST T depression or any ST T elevation which appeared or increased gradually during exercise and disappeared or decreased gradually during 4–8 min after cessation of exercise In Table VIII the findings are subdivided according to the maximal value of the ST T change into three degrees suspective (<1 mm) definite of mild degree (>1<3 mm) and definite of severe degree (>3 mm) In 61 patients (29%) a possible additional cause for ST T deviation was present In this group as well as in the total series approximately 70% developed ST T changes indicating myocardial ischaemia during exercise Frequently the ST T deviation consisted of an elevation of the ST T segment in the lead facing the infarcted area in fact this was the case in 16 of the 31 severe ST T changes Anginal pain during exercise as appears from Table III developed in 11 2% i e in only approximately 1/6 of the patients with simultaneous changes in the ST T segment Anginal pain without ST T deviation was observed in three cases The maximal value of the product HR×SBP was not significantly different in the three groups with different degrees of ST T changes

Table IV *Heart rate and systolic blood pressure during exercise in 209 patients three weeks after myocardial infarction (mean ±1 S D and range)*

	HR (beats/min)	*SBP* (mmHg)
Rest supine	80± 10 55 125	126± 18 100–215
Rest sitting	83+ 8 58–125	125± 18 95 215
Maximal work load	129± 16 90–170	170± 30 120 270

Table V *Heart rate and systolic blood pressure at maximal work load in 209 patients with myocardial infarction and different work capacity (mean±1 S D and range)*

p values indicate significance levels for differences between the groups work loads equal to 50–100–150 W

Max work load (*kpm/min*)	*n*	HR (*beats/min*)	*p*	SBP (*mmHg*)	*p*
300	93	120± 15 90–160		160± 28 120–270	
			<0 001		<0 001
600	86	132± 16 90–168		176± 25 120–260	
			<0 001		<0 1
900	30	147± 13 115–170		186± 22 150–210	

Table VI *Maximal heart rate and duration of work in different age groups (mean±1 S D and range)* p values indicate significance levels between the groups

Age (y)	n	Max HR (beats/min)	p	Duration of work (min)	p
<49	33	144± 18 105–170		9 7±3 7 2–18	
			<0 005		<0 005
50–59	87	133± 16 95–170		8 2±3 8 1–18	
			<0 005		<0 005
60–69	58	125± 17 90–155		5 4±3 1 1–13	
			<0 005		<0 02
> 70	31	115± 14 90–140		4 0±2 2 1–8	

The disturbances in cardiac rhythm and conduction observed at rest and during exercise are presented in Table IX Dysrhythmias of the listed types were present at rest in 67 patients (32%) and appeared during exercise in 106 patients (51%) Supraventricular ectopic rhythms were definitely less frequent both at rest and during exercise than were ventricular ectopics which appeared during and after exercise in 87 patients (42%) The most serious rhythm disturbance observed was one case of ventricular tachycardia lasting for 10 sec and disappearing as soon as the exercise was stopped In 7 patients on the other hand rhythm disturbances present at rest disappeared completely during work (supraventricular and ventricular ectopics in 2 and 5 cases respectively)

DISCUSSION

It is difficult to find another published series of quite similar experiences—consecutive unselected cases of all ages examined during attempted maximal exercise in the third week after AMI Kentala (8) examined 148 male patients below 65 years of age (mean 52 9) 6–8 weeks after AMI i e 3 1/2–5 1/2 weeks after discharge The patients exercised to a subjectively maximal physical work or a HR not exceeding 150/min Sanne (12) studied 291 AMI patients below 58 years of age (mean 50 4 for men 52 6 for women) 3 months after the infarction aiming through stepwise increasing work loads at a maximal performance The material of Ericsson et al (3) comprised 100 patients in the third week after infarction 16 being older than 65 years but in the

Table VII *Product heart rate×systolic blood pressure at rest and at maximal work load for all patients and maximal value in different groups according to the reason for stopping exercise (mean±1 S D)*

	N	HR (beats/min)× SBP (mmHg)×10 [a]
All patients	209	
At rest		103±25
During maximal work		224±58
Fatigue	121	234±51
Angina pectoris + dyspnoea	50	210±54
Arrhythmias + ECG changes	19	203±49

exercise aiming at a HR of only 120/min not allowing the HR to exceed 140/min Atterhog et al (1) published data from exercise tests in 12 patients aged 39–46 years 3 weeks after an anterior myocardial infarction not allowing the HR to exceed 120–130/min

As for control values special interest is attached to some recent population studies Grimby et al (4) studied 793 men 54 years of age i e all men born in Gothenburg in 1913 who were submitted to stepwise increasing work loads until a maximal physical performance Hagerup (5) studied 50-year old individuals of a population of whom 371 men and 215 women performed the exercise test of at least 6 min at 600 kpm/min

The studies quoted have demonstrated that patients after an AMI are still able to perform consid

Table VIII *Changes in ST T level indicating myocardial ischaemia provoked by exercise test three weeks after myocardial infarction*

	All cases	No other cause for ST T dev	With possible additional cause for ST T dev [a]
No ST T deviation	34	26	8
ST T deviation			
Suspective	27	19	8
Definite (>1<3 mm)	115	83	32
Definite (>3 mm)	31	20	11
Undefinable	2	0	2
Total	209	148	61

[a] *Bundle branch block 25 ventricular hypertrophy 43* digitalis 2 other causes 3

Table IX *Disturbances in cardiac rhythm and conduction during exercise test three weeks after myocardial infarction*

	Present at rest	Not present at rest but occurring during or after exercise
Supraventricular ectopic beats <10%	7	31
Supraventricular ectopic beats >10%	0	3
Atrial fibrillation/flutter	4	2
Supraventricular tachycardia	0	2
Ventricular ectopic beats <10%	31	66
Ventricular ectopic beats >10%	8	21
Ventricular tachycardia	0	1
Bundle branch block	25	3
A V block mobitz II	0	1
Total no of rhythm and conduction disturbances	75	130
No of patients	67	106

erable physical exercise The present series shows that this is true also for the majority of patients of all ages in their third week after an AMI The physical capacity of our patients being at an earlier stage after AMI and comprising all ages was probably not smaller than that of the previously published series of AMI patients but direct comparisons are not possible As would be expected the physical performance at the test was definitely larger in a population study by Grimby et al (4) of 54 year-old men of whom 95% were able to work at 600 kpm/min for 6 min and in Hagerup s (5) population study of 50-year-old individuals of whom 92% of the men and 65% of the women completing the test were able to perform the same exercise In the present study this was achieved by only 18% of the patients

In only 9% of the patients in our series was the exercise stopped by the investigator because of significant rhythm disturbances or pronounced ST T changes as discussed below This is in accordance with the experiences of Sanne (12) and Kentala (8) Anginal pain however occurred less frequently in our series and was the reason for discontinuing exercise in only 11% as opposed to 41% in Kentala s and 33–38 5% in Sanne s series In the present series by far the most common cause for stopping exercise was plain fatigue (in 58%)

HR at rest in Sanne s (12) series was significantly higher than in the population study by Grimby et al (4) In our patients with higher age and closer to their AMI the resting HR was still higher on an average but no direct comparison can be made The maximal HR during exercise in our AMI patients was for all age groups lower with a wider range and standard deviation than in the population studies and as in normal subjects (2 14) negatively correlated to age Presumably this distribution reflects different degrees of impaired myocardial function in the AMI patients on top of the general changes with age

The product of HR and SBP correlates well with myocardial oxygen consumption at rest and during exercise regardless of whether the BP is measured by a central aortic catheter or by a BP cuff (9 10) The strong correlation has also been shown in patients with coronary heart disease (7) This product was within the normal range at rest in our series and during maximal exercise there was an increase to about twice the resting values which is in accordance with the findings of Kentala (8) and Sanne (12) in their patients 1½–3 months after an AMI but less than the 4 fold increase found in both normals and in patients with ischemic heart disease but no AMI (7)

ST T deviations suggesting myocardial ischemia occurred during exercise in the great majority of our patients in most of them without simultaneous appearance of anginal pain The frequent occurrence of ischemic" ST T deviations during exercise in patients with a relatively fresh AMI is in accordance with the findings of others (1) It has been demonstrated in other studies (1 13) that such ST T deviations during exercise have often diminished or disappeared 5–6 months after an AMI

In 42% of our patients ventricular ectopic beats appeared during exercise and one patient developed a ventricular tachycardia of 10 sec duration but all rhythm disturbances disappeared spontaneously shortly after cessation of exercise Ericsson et al (3) found ventricular ectopics during and after exercise in 19% of their patients The higher incidence of ventricular ectopics in our series might be due to a higher work performance and possibly also to our series being unselected with regard to age

Serious rhythm disturbances provoked by exercise occur apparently only rarely even in patients in their third week after AMI The prognostic significance of exercise provoked dysrhythmias in

the third week after AMI is still unknown An investigation hereof and of its possible therapeutical consequences is in preparation

It is concluded that an exercise test aiming at symptom limited maximal performance when performed under controlled circumstances with certain rules for discontinuing exercise is safe in patients of all ages in the third week after an AMI Such exercise tests do not only describe the patient s physical capacity and reaction to work at the time of discharge but are also of psychological importance encouraging the patient s return to active life

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Diagnosis of Ventricular Septal Defect in Acute Myocardial Infarction without Cardiac Catheterization

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ABSTRACT Five patients with acute myocardial infarction and a systolic murmur suggestive of ventricular septal rupture or mitral regurgitation have been examined by external vascular isotope dilution curves over the chest after i v injection of ^{125}I hippuran In three patients these isotope dilution curves showed signs of left to-right shunting of blood and subsequent autopsy demonstrated the presence of ventricular septal defect In two patients there was no sign of left to-right shunting and these patients recovered

Rupture of the ventricular myocardium is a fairly common complication of acute myocardial infarction (AMI) Rupture of the ventricular septum constitutes a small proportion of the total number of ventricular ruptures (7 14) but the diagnosis of septal rupture is important because of the possibility of emergency surgical repair (2 6 12) A classical diagnostic problem in AMI is the distinction between ventricular septal defect and mitral regurgitation Both give rise to a systolic murmur which may be difficult to interpret correctly The simultaneous occurrence of both is extremely rare (4 13) Bedside demonstration of septal defect by heart catheterization has recently been described (9 10 11)

In this communication we wish to draw attention to a technique which can demonstrate septal defect in AMI and which we believe is simpler and less hazardous than other techniques described for this purpose

METHOD

The technique employed was essentially that of Folse and Braunwald (5) Briefly a scintillation detector (2 in diameter sodium iodide crystal collimation by cylindrical lead shielding extending 10 cm beyond surface of crystal) was placed over the right upper lung field of the supine patient The detector was connected to a rate meter the output of which was recorded by an ink writer giving count rate as a function of time The time constant was usually 0 5 sec and the paper speed usually 4 mm/sec

The indicator was ^{125}I hippuran (AB Atomenergi Studsvik Sweden) Bolus injections of 30–100 μCi (3–10 ml immediately washed in 10–20 ml saline) were given through a plastic catheter with its tip in a central vein Two or three injections were given at a few minutes intervals

The resulting curves were analysed as described by Folse and Braunwald (5) The interval (T) was measured between the appearance of the isotope under the detector and the time of the peak activity This peak activity (C_1) was measured as well as the activity (C_2) at an identical interval (T) after the peak activity Previous experience in our laboratory has indicated that a C_2/C_1 ratio below 40% excludes intracardiac left to right shunt (1) A ratio exceeding 50% is invariably associated with a left to-right shunt

The measurements were made in the Coronary Care Unit using portable equipment There was no special preparation of the patient Prior administration of iodine to reduce the radiation dose to the thyroid gland may be preferable but to avoid delay was not applied to the cases reported here

CASE REPORTS

Case 1

The patient a 67 year old man was admitted in Sept 1972 because of acute anterior transmural myocardial infarction Three days after admission he had an episode of three hours duration with severe chest pains and a fall in systolic BP from 150 to 90 mmHg On the following morning a systolic murmur of intermediate frequency was heard maximal at the left sternal border

An external isotope dilution curve performed four days after admission did not confirm the suspicion of a rupture of the interventricular septum the C_2/C_1 ratio being 36% (Fig 1)

In the subsequent course the murmur persisted Except a prolonged low grade fever no other complications occurred An ECG in March 1973 still showed elevation of the S–T segments in leads V_1–V_3 A chest X ray showed a heart volume of 590 ml/m² BSA the pulmonary vascular

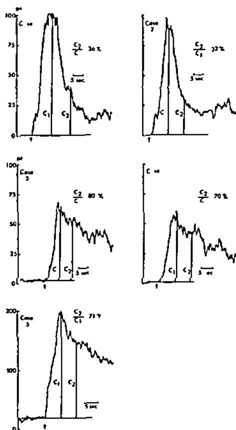


Fig 1 Radioactivity (c p s) recorded over the right upper lung field in five patients after i v injection of ^{125}I hippuran (↑) Patients 1–4 received 30 μCi ^{125}I hippuran patient 5 100 μCi Note slow disappearance of radioactivity after maximum in patients 3 4 and 5 in whom rupture of the ventricular septum was subsequently demonstrated at autopsy

markings were normal A cinefluorography showed signs of myocardial aneurysm with inverse pulsations at the upper left cardiac contour

Case 2

An 80 year old man was admitted because of a transmural anterior myocardial infarction after prostatectomy Two days after admission a systolic decrescendo murmur of medium frequency was heard maximal in the fourth inter costal space An external isotope dilution curve performed three days after admission showed a C_2/C_1 ratio of 32% (Fig 1) The systolic murmur persisted during the hospital stay but the clinical course was uneventful A chest X ray showed a normal heart volume and normal pulmonary vascular markings

Case 3

A 62 year-old woman had a bypass operation of the left femoral artery in 1970 because of arterial occlusion She had experienced angina on effort during the last few years and an ECG in 1971 showed signs of an inferior myocardial infarction Previous examinations revealed no heart murmurs

The patient was admitted in Nov 1972 after an attack of severe chest pains 16 days before On admission she was in acute distress she was drowsy dyspnoeic and the extremities were cold and cyanotic BP was 90/70 mmHg and the central venous pressure 27 cm of water ECG showed sinus rhythm signs of old inferior and recent extensive transmural anterior infarction There was a loud systolic murmur maximally in the third and fourth left interspaces PCG showed a holosystolic murmur of crescendo–decrescendo type

Treatment was started with digoxin and diuretics i v heparin low molecular weight dextrane and chlorpromazine in i v drip The condition improved she was more alert and peripheral perfusion improved

Two days after admission an external isotope dilution curve showed signs of a large left to right shunt with a C_2/C_1 ratio of 80% (Fig 1) Thus the diagnosis of ventricular septal rupture was confirmed and an emergency operation with closure of the rupture was considered However operation was not performed because of the general condition of the patient and her advanced arteriosclerotic vascular disease Fourteen days after admission her condition deteriorated with rising temperature drowsiness increasing dyspnoea and signs of right heart failure and she died three days later

The necropsy revealed advanced general arteriosclerosis The heart weight was 400 g the right ventricle was dilated The left anterior descending coronary artery was occluded 3 cm from the origin and a septal rupture with a diameter of 2 cm was found within a large anteroseptal infarction in a healing stage The mitral valve was normal

Case 4

A 59 year-old man had an attack of severe precordial pain one week before admission because of chest pains of 12 hours duration ECG showed acute inferior myocardial infarction There was no heart murmur On the following morning a loud high frequency systolic murmur of crescendo–decrescendo type was heard from the apex to the left sternal border

Because of the location of the infarction there was a suspicion of mitral regurgitation caused by a rupture of a papillary muscle However the external isotope dilution curve performed two days after admission showed a C_2/C_1 ratio of 70% (Fig 1) The patient had recurrent chest pains and he was dyspnoeic The systolic BP was 90–70 mmHg and the central venous pressure 23–27 cm H_2O He died on the third day of admission

Necropsy showed a large myocardial infarction in the posterior heart wall and the upper posterior part of the interventricular septum There was a rupture of the septum and of the posterior heart wall with pericardial tamponade

Case 5

A 68 year-old woman was admitted in Sept 1972 because of retrosternal pain of four hours duration on the day before admission She was dyspnoeic and the skin was cold and clammy There were pulmonary rales but no

heart murmur ECG showed transmural anteroseptal infarction left anterior hemiblock and RBBB SGOT increased from 160 to 576 U and LDH was 1750 U with increase of iso-enzymes 1 2 and 5

On the second day of admission auscultation revealed a systolic murmur of high frequency along the left sternal edge maximal in the third and fourth left intercostal space and an external pulmonary vascular isotope dilution curve was performed The result is shown in Fig 1 indicating a left to right shunt with a C_2/C_1 ratio of 71% During the following days her condition deteriorated and she died eleven days after admission

At necropsy the heart weight was 510 g There was an occlusion of the left anterior descending coronary artery and an extensive infarction of the septum apex and inferior wall of the left ventricle A rupture 2 cm in diameter was found in the middle part of the interventricular septum The mitral valve was normal

DISCUSSION

Heart catheterization and angiography are the current methods for confirming or refuting the clinical diagnosis of rupture of the ventricular septum (3 10 12 15) Angiocardiography which will be needed before an operation to define the size and location of a septal defect is obviously not suitable as a screening procedure due to its hazards and inconvenience Right heart catheterization can yield some information about the site of a left to right shunt by the step up in blood oxygen saturation

We feel however that external pulmonary vascular isotopic dilution curves as described in this report provide a simpler means of showing the presence or absence of a left to right shunt A variety of factors can influence the descending limb of the pulmonary vascular isotope dilution curve In situations with a prolonged transit time of blood in the thorax (e g low cardiac output regurgitation large intrathoracic blood volume) both ascending and descending limbs will be slow (5 8 16) In such cases the C_2/C_1 ratio will still be normal Our limited experience suggests that external pulmonary vascular isotopic dilution curves can be informative in the differential diagnosis between rupture of the ventricular septum and mitral regurgitation in acute myocardial infarction

ACKNOWLEDGEMENT

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Effects of Long-term Treatment with Procaine Amide

A Prospective Study with Special Regard to ANF and SLE in Fast and Slow Acetylators

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ABSTRACT During 1970–75 a total of 42 patients have been subjected to long term treatment with procaine amide (PrA) because of different cardiac arrhythmias and have been observed up to over 5 years Among these patients 35 (83%) developed a significantly increased titer of ANF and of these, 12 patients (29%) developed a ' classical ' drug induced SLE syndrome In the SLE group all but 2 improved rapidly after cessation of PrA, and the ANF titer decreased continuously but slowly in both groups Acetylation test with sulphamidine and/or isoniazid in 11 patients among the SLE cases showed 8 slow and 3 fast acetylators Among 12 patients who also had received PrA for a long time, but had not shown any signs of an SLE syndrome, there were 10 fast and 2 slow acetylators

Several authors have shown that procaine amide (PrA) may cause an autoimmunity like state which is similar to systemic lupus erythematosus (SLE) except that the kidneys are hardly ever involved in the PrA induced syndrome (4 5 16 17 25) Similar reactions have been described for other drugs such as hydralazine isoniazid penicillamine phenytoin and others but the frequency of abnormal immunological findings and symptoms related to autoimmunity seems to be much higher during therapy with PrA (3 7 9 38) In 1973 Perry (31) showed that of 25 patients who had gone through a hydralazine induced SLE all except one belonged to the slow acetylator phenotype—i e these patients had a low level of the polymorphous hepatic acetyl transferase activity as measured with a sulphamidine acetylation test (33) PrA seems to be partly metabolized in the liver by the same enzyme system as hydralazine and therefore the same relationship between slow acetylators and SLE syndromes might be suspected also in PrA treated patients

The present study was performed in patients on long term treatment with PrA to evaluate 1) the relation between total intake of PrA and the development of increased antinuclear factor (ANF) titer LE cells and SLE syndrome and 2) the relationship if any between the appearance of ANF or SLE syndrome and the acetylation phenotype tested with sulphamidine and/or isoniazid (INH)

MATERIAL

Forty two patients (30 men with a mean age of 61 range 31–74 and 12 women with a mean age of 63 range 42–75) were all treated with PrA for three months or longer because of cardiac arrhythmias The diagnosis in 28 patients was ventricular fibrillation and/or tachycardia and was based on a coronary heart disease (CHD) in 27 patients and on myocardial sarcoidosis in one patient Multiple ventricular extrasystoles were diagnosed in 11 patients 9 of whom had CHD and 2 had a diagnosis of non specific cardiomyopathy Three patients with rheumatic heart disease (RHD) were treated because of supraventricular tachycardia The average duration of treatmen with PrA in the whole group was 21 months (range 3–60) All patients started their treatment during hospitalization The dosage of PrA was adjusted according to the effect on the arrhythmias and in 21 patients guided by plasma concentration measurements (19 21)

With only one exception no patient was treated with other known SLE inducing drugs during the PrA treatment and none had ever experienced a drug-induced SLE Two patients were treated with oestrogens because of prostatic neoplasm Out of 6 patients with systemic hypertension 5 were treated with diuretics (thiazides) and one (M5 Table I) received a small dose of hydralazine (10 mg t i d) One patient (F4 Table I) with RHD also showed signs of chronic thyreoiditis (Mb Hashimoto) but her ANF was negative before treatment with PrA

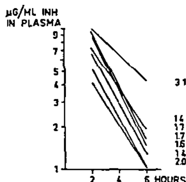


Fig 2 Plasma half-life of isoniazid in 7 PrA treated patients

females (range 42–72 years) and 40 males (range 24–81 years) The method has been described by Price Evans (33 35) and the percentage of acetylated sulphamidine in *both plasma and urine was determined From the beginning* it was clear that other medication might disturb the values because of non specific influence during measurements Therefore patients who received large amounts of a drug which on a chemical basis could be suspected to interfere with the determinations were excluded

The distribution of the plasma values did not show a clear bimodality However Fig 1 shows that in *urine* there is a clear bimodality and a dividing line around 70% of acetylation In this material 39% were fast and 61% slow acetylators This agrees well with another Swedish study on this subject (14) Among 14 females only 3 were fast and 11 slow acetylators Among 40 males 18 (45%) were fast and 22 (55%) slow acetylators This normal material comprised only inborn Swedish natives Because of the uncertainty of the sulphamidine plasma acetylation it was decided to try another test drug for the determination of the phenotypes INH (Tibinide®) was chosen and given orally in a dose of approximately 10 mg/kg The plasma concentration of INH was then determined 2 4 and 6 hours after the administration The determinations were carried out according to Lever (28) and the plasma half life of INH was obtained graphically from a semilogarithmic plot of the concentration against time (Fig 2) and 2 1 hours was chosen as the dividing value (14)

RESULTS

The pretreatment ANF titer was known in all but 7 patients As shown in Figs 3 and 4 a total of 35 patients developed a significantly increased titer of ANF with maximal titers ranging from 1 64 to 1 16384 In most of the patients there was a clear relation between total intake of PrA and ANF titer As shown by others (22) the ANF titer increased during continued therapy The patients were treated on an average during 4 months with about 400 g PrA before the ANF titer became positive The LE cell test was regularly positive in patients with ANF titers above 1 256 but was occasionally positive in patients with lower titer (Fig 5) The mean titer in the group with SLE was about 1 1024 and in the group without the SLE syndrome about 1 256 There were no differences in the immunofluorescent patterns between the two groups In all cases with maximum titers exceeding 1 128 the homogenous or peripheral type of pattern was obtained and all cases with titers 1 128 or lower showed the speckled type even with undiluted serum In most of the high titered sera the homogeneous or peripheral pattern was replaced by a speckled pattern after sufficient dilution This could occur after one or several dilution steps as a rule later or not at all with the most high titered sera In most but not all the patients increasing or decreasing titers were associated with a speckled pattern in undiluted serum even in those whose

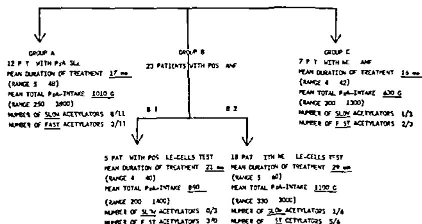


Fig 3 Forty two patients on long term treatment with procaine amide

Table I *All patients who developed SLE during therapy with procaine amide*
CHD=coronary heart disease RHD=rheumatic heart disease INH=isoniazid test M=male F=female

Pat no	Diagnosis	Total intake of PrA (g)	Max ANF titer	Dominating symptoms	Initial and max ESR (mm)	Sulpha or INH acetylation
M1 1910	CHD	800	1/8 192	Arthralgia pericarditis	2–35	Slow (sulpha)
F1 1904	Sarcoidosis	1 300	1/1 024	Myalgia fever	20–40	Fast (INH 1 4 h +sulpha)
M2 1914	CHD	700	1/512	Arthralgia fatigue	4–18	Slow (sulpha)
M3 1904	CHD	550	1/16 384	Myalgia fever	30–80	Slow (sulpha)
M4-1903	CHD	320	1/256	Polyarthritis fever	40–100	Slow (sulpha)
F2 1903	CHD	350	1/2 048	Arthralgia fatigue	5– 9	not done (†)
M5-1908	CHD	250	1/1 024	Pleurisy fever	20–78	Slow (sulpha)
F3 1923	Cardiomyo pathy	1 500	1/64	Arthralgia fever	4– 6	Fast (INH 1 7 h)
F4-1902	RHD	650	1/1 064	Arthralgia fatigue	6–18	Slow (sulpha)
F5 1913	CHD	1 200	1/128	Skin rash myalgia	8–25	Slow (sulpha)
F6-1922	RHD	850	1/1 024	Polyarthritis fatigue	30–45	Slow (sulpha)
F7 1912	RHD	3 800	1/2 048	Polyarthritis fever	15–45	Fast (INH 1 7 h +sulpha)

METHODS

Control procedures

After starting treatment with PrA the patients were controlled at comparatively short intervals—usually not longer than 3 months At every check up they were questioned for possible SLE symptoms (joint or muscle pains fever continuous chest pains or sustained fatigue) Determinations of Hb leucocytes ESR proteinuria ANF and often LE cells and immunoglobulins were made If ANF rose to 1/128 or more or if suspect SLE symptoms appeared the patients were examined more extensively i e with a chest X ray hematological examination with thrombocytes reticulocytes differential count of leucocytes Coombs test rheumatoid factors (Rose Waaler and latex fixation test) and plasma electrophoresis with special examination of the C3 and C4 complement factors were also performed The following ranges were considered normal IgG 7 0–16 0 g/l IgA 1–3 g/l IgM 0 5–2 0 g/l C3 or βlC 60–140% C4 or βlE 60–140% If the patients stopped treatment with PrA a check up every half year with the above mentioned routine measurements was made in most cases

SLE criteria

The patients were accepted as having a PrA induced SLE if all of the following criteria were present I) A significantly raised ANF titer (see below) II) Positive LE cell test (see below) III) Typical symptoms and objective clinical findings such as arthralgia arthritis pleurisy pericarditis pulmonic infiltration skin rashes diffuse myalgias fever continuous fatigue IV) The symptoms and/or objective clinical findings disappeared or diminished markedly after withdrawal of PrA

Immunofluorescent assay for antinuclear factors (ANF)

Indirect immunofluorescent technique was used according to the method described by Alexander et al (1) with slight modification Leucocytes in unfixed films of fresh capillary blood were used as nuclear substrate Fluorescein isothiocyanate conjugated sheep antiserum to human IgG (including light chains) diluted 1 30 was applied as indicator The optical equipment was a Leitz Ortholux microscope with Osram HBO 200 mercury lamp dark field condensor excitation filter BG 12 3 mm and secondary filter OG 1 2 5 mm The magnification used was ×312 Each serum was tested undiluted and diluted 1 16 When positive titrations to the endpoint were made The titer of a serum was estimated as the greatest dilution giving nuclear fluorescence *Titers of 1 16 or less* were said to be negative Fourfold titer changes were judged as significant The fluorescence staining pattern was characterized as homogenous peripheral or speckled

LE cell tests were made by the method described by Hargraves et al (15) Only typical LE cells with or without rosettes were accepted for a positive test

Acetylation test

Acetylation tests were performed in 23 patients who had been treated with PrA The control group consisted of 54 consecutive hospitalized patients from the Cardiology Ward—49 of them had a diagnosis of CHD 2 of acute pericarditis 1 of borderline hypertension 1 of anemia and 1 of RHD Their mean age was 59 and there were 14

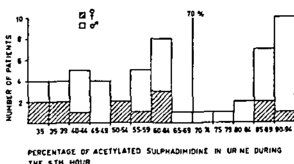


Fig 1 Control group Distribution of acetylation phenotypes in 54 hospitalized patients from the Cardiology Ward

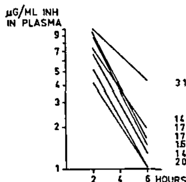


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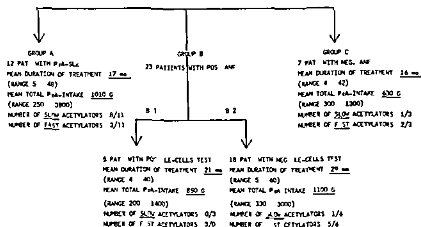


Fig 3 Forty two patients on long term treatment with procaine amide

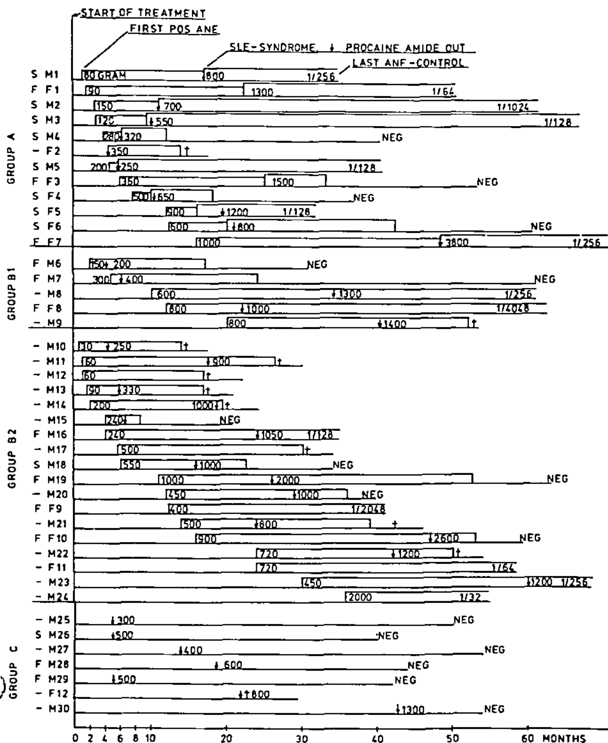


Fig 4 Longitudinal presentation of all PrA treated patients See also Fig 3 *S*=slow acetylator *F*=fast acetylator Each small dot represents an ANF titer determination [80 ↓800 1/256]—this sequence shows that after intake of 80 g PrA the patient developed a significantly raised ANF titer and after 800 g a PrA induced SLE syndrome occurred Arrow indicates withdrawal of PrA the last figure indicates the ANF titer at latest check up † indicates death

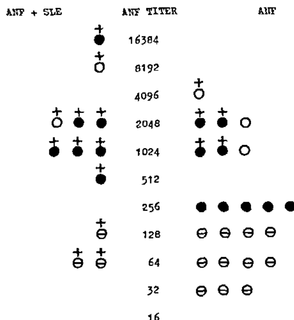


Fig 5 The 35 cases with positive ANF with (left) and without (right) SLE syndrome *Maximum ANF titers and* immunofluorescent staining patterns (● homogenous ○=peripheral ⊖=speckled) + =LE cells found

maximum titered sera produced the homogeneous or peripheral pattern

Group A (Figs 3 4 and Table I)

Twelve patients 7 of 12 females and 5 of 30 males (29%) developed a clear cut drug induced SLE with typical symptoms (arthralgia 8 polyarthritis 3 pleurisy 4 pericarditis 2 diffuse myalgia 2 pulmonic infiltrations 2 and skin rash 1) All had fever and complained of fatigue (Table II) The onset was abrupt—days—in 2 patients (M5 and F4) but more insidious in the remainder with symptoms mostly developing during 3–4 weeks Usually ESR increased and in 4 cases (M1 M4 M3 and F6) mild leucopenia (<3 000 mm³) was found Thrombocytopenia (<130 000 mm³) was present in only one case (F6) Coombs test was negative in all the 10 cases in whom it was performed No case of proteinuria was noted

Immunoglobulins were increased in 4 patients (F3 IgG 16 g/l M4 IgG 17 g/l F5 IgA 3 3 g/l F6 IgM 5 g/l) and all were polyclonal Two patients had very low IgA values (F2 0 2 g/l and F4 0 07 g/l) In 2 patients C4 was low (F4 15% and F7 27%) and remained low six months after treatment had been stopped In one patient (F7) it became normal (100%) after one year and this patient also had symptoms for almost a year although gradually decreasing in intensity

In 4 patients (M1 M4 F4 and F6) RhF (Rose Waaler (rabbit) and acryl fixation test (human)) became positive and in 2 of these patients (M4 and F4) the tests were again negative 6 months after withdrawal of PrA

The ANF titers decreased very slowly after cessation of PrA and in only 4 patients (M4 F3 F4 and F6) was the titer negative after 17–30 months One patient (M3) still has a positive titer of 1 128 5 years after cessation of treatment The LE cell test was taken irregularly after cessation of PrA but it became *negative in most patients after one year*

Acetylation tests were done in 11 patients and 8 were slow and 3 fast acetylators This distribution does not differ from that in the controls All the 3 fast acetylators were females (F1 F3 and F7) and had shown signs of chronic progressive disease (cardiomyopathy sarcoidosis RHD) All the slow *acetylators but one (F5) had a diagnosis of CHD*

Of 4 patients with PrA induced SLE 3 (M5 F1 F7) developed toxic reactions to quinidine and one (M1) to phenytoin after cessation of PrA treatment These reactions consisted of muscle pains fever and in 2 cases (M1 F1) of pleurisy These symptoms disappeared after withdrawal of the drug One patient in this group has died from CHD during the observation period

Table II *Twelve patients without PrA SLE but long term treated with procaine amide and tested for their acetylation phenotype*

Abbreviations as in Table I

Pat no	Total intake of PrA (g)	Max ANF and LE cells	Acetylation phenotype
M 6	200	1/1 024 LE cells	Fast sulpha
M 7	400	1/2 048 LE cells	Fast sulpha
F 8	1 000	1/4 096 LE cells	Fast sulpha
M 16	1 050	1/256	Fast sulpha
M 18	1 000	1/32	Slow sulpha
M 19	2 000	1/32	Fast INH (1 6 h)
F 9	1 000	1/2 048 not done	Fast sulpha
F 10	2 600	1/1 024	Fast sulpha
M 24	3 000	1/32	Fast sulpha
M 26	500	Negative	Slow sulpha and INH (3 1 h)
M 28	600	Negative	Fast INH (1 4 h)
M 29	500	Negative	Fast INH (2 0 h)

Group B

As shown in Figs 3 and 4 23 patients developed a positive ANF titer but no signs of an SLE syndrome They were on an average treated for a longer period than the SLE group (Fig 3) All these patients showed normal blood values and none developed proteinuria The ANF titer also remained positive for a longer period than in the SLE group Five patients developed a positive LE test (group B1) and all 3 tested for their acetylation phenotype were fast acetylators Among the patients with negative LE test (Group B2) 6 were tested and only one (M18) was a slow acetylator but this patient had a borderline urine sulpha acetylation value of 68% Nine patients in this group have died during this observation time (Fig 4) 8 from CHD and one because of malignancy

Group C

This group comprised 7 patients and they needed treatment on an average during a shorter period (Fig 3) One of these patients (M26) developed SLE suspect symptoms with arthralgias after 2 months treatment but ANF was negative and remained so one month later when treatment was stopped This patient was a slow acetylator but the other two tested in the group were fast acetylators (Fig 4) One patient died from CHD

DISCUSSION

This study and several others show that development of positive ANF titer is more a rule than an exception during long term treatment with PrA (5 17 22 26) In the first published prospective study by Blomgren et al (4) 8 of 16 patients developed positive ANF titers and 4 a PrA induced SLE Kosowsky et al (22) found that all their 30 patients treated with PrA for at least one year developed a positive ANF In our study 7 patients did not develop a positive ANF even when they were treated for more than one year but our standard dosage 1–2 g/day is lower than that used by Kosowsky et al These authors observed a similar high rate of SLE syndrome as in the present study

In most patients with a drug induced SLE syndrome the clinical condition improves rapidly after withdrawal or reduction of the drug but in a few cases symptoms may persist for a long time and disappear only after treatment with corticosteroids (14 16) Also in this study 2 patients (F1 F7) had persisting symptoms Patient F1 was given a small dose of prednisolone (10 mg/day) during the PrA administration despite this she developed an SLE syndrome and her symptoms continued for several months after withdrawal of PrA Five months later the patient also developed a toxic reaction to quinidine with high fever myalgia and pleurisy but the ANF titer remained unchanged Patient F7 had symptoms of diminishing intensity for almost a year and the complement factor concentration did not normalize until after a year

No typical immunofluorescent staining pattern was found in the patients who developed the SLE syndrome but the staining pattern was found to correlate well with the titer levels The speckled type of fluorescence is generally ascribed to an extractable nuclear antigen (24 36) and is common in disorders like Sjogren s disease and scleroderma whereas the homogeneous and peripheral types, caused by nucleoprotein and DNA respectively are more often found in SLE (2 8 12 13) The significance of the staining pattern has been questioned by others (10 18, 37) and the influence of serum dilution has also been emphasized (10 37) This investigation suggests that the immunofluorescent pattern is more a question of quantity in the case of PrA induced SLE or ANF In highly ANF active sera the homogenous or peripheral patterns dominate the fluorescent picture This masking of the more discrete speckled pattern disappears with decreasing total antibody titer

The mean maximum ANF titer in the SLE group was found to be somewhat higher than in the group without the SLE syndrome (Fig 5) although 3 of the SLE patients had titers of 1 64–1 128 and 7 patients without SLE had titers of 1 1024–1 4096 The ANF titer could not be used as a sign of the development of SLE symptoms

Sera from 3 patients (M3 M7 M8) were fractioned with an electrophoretic separation in agarose gel and the ANF titer was determined in each fraction In every fraction positive titers of ANF were found which could indicate that the ANF activity is spread all over the γ globulin area as described also by Gonzales and Rothfield (13)

The explanation of the biochemical background to these drug induced phenomena is still lacking but some interesting and possibly fundamental findings are discussed Blomgren et al (5) showed that ANF associated with PrA treatment appeared to be

restricted to antibodies with specificities for denatured or single stranded desoxyribonucleoprotein (DNA) In the spontaneous SLE cases the antibodies (ANF) usually react only with native DNA and usually bind complement *This difference in* specificity might explain why the kidneys never or very seldom are grossly involved during a PrA induced SLE (10 23 38 39)

Lorand et al (29 30) and later Tan (38) showed that hydralazine and INH can be firmly attached to DNA but there is no strong evidence that these complexes are more antigenic than native DNA Blomgren et al (5) bound PrA to denatured DNA but found no difference in antigenic strength in relation to only denatured DNA Tan (38) observed that DNA protein complexes treated with trypsine still reacted with serum from a patient with idiopathic SLE but not with serum from a patient with a hydralazine induced SLE and he concluded that the DNA protein complex was antigenic only in the drug induced SLE case Lorand (29) pointed out that drugs with free primary amino groups or hydrazides couple with glutamine residues of proteins e g fibrin and fibrinogen and that this reaction is catalyzed by various transamidating systems e g fibrinoligase (activated factor XIII in the coagulation system) it might be suspected that also PrA can react within this system

A low acetylation capacity of the liver seems to make patients treated with hydralazine more prone to drug induced SLE We therefore assumed this might be the case also for PrA This has lately been proposed by others too (20) Among our patients with PrA induced SLE 8 were slow and 3 fast acetylators There is thus a risk even for fast acetylators to develop a PrA induced SLE but this seems to be the case only after a high total intake and especially in patients with other chronic diseases (Fig 4 Table II) Perry et al (32) also showed that after long term treatment with hydralazine the fast acetylator group contained an increasing number of SLE cases Among the patients who did not develop SLE in the present material 12 were tested with an acetylation test and 2 were found to be slow and 10 fast acetylators This result differs significantly from the control group ($p<0\ 01$) if the ratio should be constant with all the possible 30 patients of which 10 had died

In the SLE group there were 7 females a clear overrepresentation with regard to the sex distribution in the whole material (♀ 12 ♂ 30) (0 05 $>p>0\ 01$) Another remarkable finding in regard to the control group was that almost 80% of the females were slow acetylators as against 55% of the males (Fig 1) but our material is still too small for firm conclusions In this connection it is of interest that Reidenberg and Martin (34) found the vast majority of 14 female patients with spontaneous SLE to be slow acetylators The assumed normal acetylation phenotype distribution among females being just 80% slow and 20% fast acetylators may also help to explain why Bottiger (6) found that females were 3 times more prone to develop drug side effects even after allowance for differences in consumption

Eleven patients in this study died during the observation period Ten of these deaths could be ascribed to CHD whereas one patient died from cancer There are reasons to believe that a great percentage of these deaths are caused by ventricular fibrillation (27) The benefit of long term treatment wiht antiarrhythmic agents is controversial (22 40) and must be weighed against the side effects—the present study has shown a high complication rate in patients on long term treatment with PrA

If long term treatment with PrA is considered an acetylation test should be performed and slow acetylators should be excluded from prolonged PrA therapy unless there are exceptional indications to the contrary

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Verapamil in the Treatment of Atrial Tachycardia with Block

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ABSTRACT Atrial tachycardia (A T) with block is easily converted as a rule to sinus rhythm by DC shock but the recurrence rate is high So far drug treatment of this arrhythmia has been unsatisfactory There is no drug of choice in the treatment of A T with block We report on the effect of verapamil on this arrhythmia in 14 patients Ten of them reverted to sinus rhythm, but A T with block later recurred in 4 The best effect was found in patients with no or only minimal heart enlargement and in patients with short duration of the arrhythmia The effect of verapamil on A T with block may support the concept of this arrhythmia being due to atrioventricular (A V) nodal reentry, as the main effect of verapamil is a prolongation of the effective refractory period of the A V node

Verapamil introduced as a coronary vasodilator was soon shown to possess antiarrhythmic properties especially in patients with supraventricular tachyarrhythmias (10 13) It has been shown to be effective in the treatment of atrial or junctional tachycardias and to suppress the ventricular rate in patients with atrial flutter and fibrillation (14)

Electrophysiological studies have shown that it may depress the spontaneous activity in the isolated rabbit sinoatrial (S A) node (17) In the dog however there was no effect on the sinus rate or on the impulse conduction through the atria the His Purkinje system and the ventricles (7) The most pronounced effect was observed in the atrioventricular (A V) node where the effective refractory period was prolonged with a marked reduction in the conduction velocity within the A V node According to Paes de Carvalho et al (11) A V action potentials are due entirely or almost entirely to a slow component which seems to be related to an inward channel which might be used by Ca^{++} ions or Na^{+} and Ca^{++} ions (3 12) Verapamil which has been classified as a calcium antagonistic inhibitor of the electromechanical coupling process (5) reduces the influx of Ca^{++} ions into the myocardial cell (6) and thus affects refractoriness and conductivity of the A V node

The lengthening of the refractory period of the A V node may explain the pharmacological effect of verapamil on atrial tachyarrhythmias as this blocking effect may prevent A V nodal reentry which is thought to initiate and perpetuate A T (4) The suppression of conduction through the A V node will further tend to slow ventricular rate in atrial fibrillation or flutter

Atrial tachycardia (A T) with block has been supposed to be due chiefly to digitalis intoxication (8) In a recent study (15) of 31 patients with A T with block we found that none of them fulfilled Lown s criteria for considering digitalis to be the common etiological factor in A T with block Most of the patients had serum digitoxin concentration within the therapeutic range of 15–25 ng/ml Only 7 patients had somewhat higher concentrations In the study mentioned we found that 2 patients who were severely ill during the period of A T with block responded to treatment with verapamil in a dose of 80 mg every third hour One of them returned to sinus rhythm and the other to nodal rhythm which later changed to sinus rhythm The present study was designed to investigate the long term effect of verapamil in this arrhythmia

MATERIAL AND METHODS

During the recent 2 years (Jan 1 1973–Dec 31 1974) we have treated 22 patients with A T with block Most of them had valvular heart disease and many had been operated on (Table I) Some of the patients had had A T with block for several years while in others the tachycardia was of recent origin All patients but one were receiving

Table I *Findings on admission*

HV=heart volume AF=atrial flutter A T=atrial tachycardia with block A V=atrioventricular S-A=sinoatrial PM=pacemaker

Pat no	Sex	Age (y)	Diagnosis	Relative HV (ml/m²)	Previous rhythm	Duration of A T	Rhythm on admission
1	♀	58	Mitral valve replacement	1 020	AF	4 d	A T a 270–v 65
2	♀	37	Struma non toxic	390	A T	22 y	A T a 270–v 80
3	♂	36	Scleroderma A V block	500	A T	Parox 4 mo	A T a 270–v 135
4	♀	68	Aortic valve replacement	1 000	AF A V block	14 d	A T a 220–v 110
5	♀	60	Mitral+aortic valve replacement	650	A T	2 mo	A T a 180–v 90
6	♂	57	Angina pectoris	525	A T	Parox 8 y	A T a 180–v 80
7	♂	49	Coronary bypass	430	Sinus	14 d	A T a 300–v 150
8	♂	56	Mitral valve replacement	760	Sinus	2 mo	A T a 250–v 62
9	♀	33	Atrial septal defect operat	430	Sinus	5 y	A T a 230–v 115
10	♀	66	Mitral valve replacement	720	AF	2 y	A T a 230–v 115
11	♀	57	Mitral comm	510	Sinus	Parox 2 y	A T a 270–v 135
12	♂	81	Coronary heart disease	540	Sinus	Parox 5 y	A T a 250–v 80
13	♀	48	Mitral comm	670	Sinus	Parox 5 y	A T a 180–v 90
14	♂	63	Coronary heart disease	560	S A block AF	1 mo	PM a 180–v 90
15	♀	52	WPW syndrome	460	AF	Parox 30 y	PM a 280–v 140
16	♂	76	Myocardial infarction	740	A V block Pacemaker	3 mo	A T a 240–p m 70
17	♀	59	Mitral valve replacement	860	AF	2 mo	A T a 220–v 90
18	♂	53	Mitral valve disease	1300	AF	1 mo	A T a 160–v 80
19	♂	*71*	*Myocardial infarction*	*800*	*AF* Pacemaker	*3 y*	*A T a 250–p m 70*
20	♀	52	Atrialseptal defect operat	430	Sinus	1 mo	A T a 190–v 190
21	♀	60	Aortic valve replacement	410	Sinus	9 mo	A T a 230–v 115
22	♀	42	Mitral comm	460	Sinus	1 y	A T a 240–v 120

digitalis (Table II) The serum digitoxin concentrations in 20 patients were between 6 5 and 25 ng/ml Only one patient had a somewhat high concentration of 35 ng/ml (therapeutic range 15–25) The serum potassium concentration was normal in all patients except one whose K concentration was 3 2 mEq/l Digitalis treatment was continued in all patients

Verapamil was given to 14 of the patients usually every third hour in a dose of 40 mg If there was no effect the dose was increased to 80 mg every third hour Patients with a ventricular rate below 90/min were usually not treated with verapamil

Electroconversion of A T with block was carried out in 5 patients Two patients were treated with quinidine Six patients had pacemaker implantation due to S A or A V block

RESULTS

Six patients were treated with pacemaker for S A or A V block (Table II) One of them achieved sinus rhythm which is still maintained Three patients still have A T with block one has atrial fibrillation and one has S A block Of the 6 patients in whom electroconversion was carried out two reverted to sinus rhythm One of them is maintained on quinidine in the other atrial fibrillation has developed in spite of treatment with verapamil

Of the 14 patients treated with verapamil 6 are maintained in sinus rhythm 5 still have A T with block, while 3 have atrial fibrillation (Table II) The treatment with verapamil has usually not been continued on a long term basis only 2 patients are still using verapamil One of these patients is mentioned below (no 21)

The effect of verapamil may be illustrated by patient 20 who had A T with block following operation for an atrial septal defect Her A T was treated with DC shock with temporary conversion On admission to the Medical Department on March 15 1974 at 15 h she had A T without block with a rate of 190 (Fig 1) She was given verapamil 40 mg every third hour One hour later the ventricular rate had fallen to 115 and ECG showed A T with block 230 115 During continued administration of verapamil *A T with block reverted to sinus rhythm on the following morning* (Fig 2)

Patient 21 had A T following aortic valve replacement *On admission she had A T with block 230 115 Shortly* afterwards she had A T without block she was then given verapamil 5 mg i v followed by 80 mg orally (Fig 3)

Table II *Previous and present treatment*

Abbreviations as in Table I

Pat no	Digitoxin mg/d	Digitoxin Serum conc (ng/ml)	Potassium (mEq/l)	Pace maker	Electroconversion	Quinidine (g/d)	Verapamil (mg/d)	Rhythm following treatment	Present rhythm	Follow up	Verapamil cont
1	0 05	16 5	3 6	–	–	–	40×3 (4 d)	A T	A T	1 mo	No
2	0 1	–	4 5	–	–	–	–	A T	A T	1 y	No
3	–	–	4 5	+	–	–	40×8	PM Sinus	Sinus	1 mo	No
4	0 1	35	4 4	+	–	–	80×6	PM AF	AF	3 mo	No
5	0 1 5 d/w	17	4 0	–	–	–	–	A T	A T	5 mo	Died
6	0 1 5 d/w	19 5	4 3	–	–	0 60×2	80×8 (3 d)	Sinus	Sinus	7 y	No
7	0 1 5 d/w	10	3 8	–	–	–	80×8 (3 d)	Sinus	Sinus	1 mo	No
8	0 1	21	4 1	–	+	–	40×4	A T	A T	6 mo	No
9	0 05	8	3 5	–	–	–	40×8	Sinus	Sinus	1 y	Yes
10	0 1 6 d/w	15	3 7	–	+	–	–	Sinus	AF	3 y	No
11	0 1 5 d/w	25	4 0	–	–	–	40×8	Sinus	AF	1 y	No
12	0 1 5 d/w	12	4 2	–	+	0 40×2	–	Sinus	Sinus	1 y	No
13	0 1 5 d/w	17	5 1	+	+	–	40×4	PM Sinus	A T	1 mo	No
14	Lanoxin 0 25	–	4 1	+	–	–	80×4	PM S A block	S A block	2 mo	No
15	0 05	6 5	4 2	–	–	–	80×4	A T	A T	1 mo	No
16	0 1 5 d/w	–	5 0	+	–	–	–	PM A T	A T	3 mo	No
17	0 05	10 5	4 1	–	–	–	–	A T	A T	1 mo	No
18	0 1 5 d/w	21 5	4 2	–	–	–	–	A T	A T	1 mo	No
19	0 1 5 d/w	20 5	4 4	+	–	–	–	PM A T	A T	1 mo	No
20	0 1 5 d/w	17 5	3 2	–	+	–	40×8	Sinus	Sinus	1 mo	Yes
21	0 1 5 d/w	14	4 5	–	–	–	80×7	Sinus	A T	6 mo	No
22	0 1 6 d/w	25	4 5	–	+	–	40×6	Sinus	A T	8 mo	No

After 1 hour her heart rate had fallen to 105 ECG showed A T with block 210 105 During further administration of verapamil 40 mg every third hour her A T with block converted to sinus rhythm Her A T with block has however returned and it has not been possible to maintain sinus rhythm During verapamil treatment in a dose of 80 mg×8 she sometimes has increasing block up to 6 1 of 8 1 The dose has therefore been reduced to 80 mg×7 On this treatment her ventricular rate is usually maintained around 90 with varying A V block

Patients with S A block seem to need smaller doses of verapamil as illustrated by patient 13 who had been operated on for mitral stenosis and had S A block varying with A T with block To prevent attacks of A T with a ventricular rate up to 160 she was given verapamil in a dose of 40 mg As shown in Fig 4 her tachycardia was suppressed with this dose but reverted after 14 hours She was therefore given verapamil 40 mg 3 times a day which was able to prevent recurrence of her atrial tachycardia

Factors which might influence the effect of verapamil treatment on A T with block are illustrated in Figs 5 and 6 Fig 5 concerns the effect of heart size As will be seen all patients who are maintained in sinus rhythm following treatment with verapamil had a normal or only slightly increased heart size while most of the patients who did not respond to treatment had enlarged hearts though there were three with a normal heart size There is thus no sharp distinction between patients with small and large hearts although generally the effect is better in those with small hearts

The influence of the duration of the arrhythmia on the effect of verapamil treatment is illustrated in Fig 6 As will be seen there is again no sharp distinction between short and long duration of arrhythmia Five of the 6 patients who responded to treatment had A T with block of short duration while 5 of the 8 who did not respond to treatment had arrhythmia of long duration

DISCUSSION

A T with block is a rare form of atrial arrhythmia It is usually found in organic heart disease It has been considered to be caused by digitalis intoxication and potassium depletion and it has been found to bear a grave prognosis (8) In a previous report (15) we were not able to confirm the evidence for digitalis intoxication and the prognosis in our patients was better than usually believed as only one patient died during the observation period The present report confirms our previous impression We found no signs of digitalis intoxication in these patients Only one patient had a somewhat high serum digitoxin concentration and only one had a somewhat low potassium concentration The prognosis was also good in the present report as only one patient died and this death followed an operation

The mechanism for A T has not been elucidated According to Bigger and Goldreyer (4) it may be considered to be due to a reentry mechanism employing the A-V nodal tract The block is thought to be functional due to a rapid atrial rate of more than 200/min It might therefore be conceivable that agents which are able to prolong the refractory period of the A V node may be effective in terminating this arrhythmia

The present study was carried out to investigate the effect of verapamil on A T with block The most pronounced effect of verapamil is a marked reduction of the conduction velocity within the A-V node and a pronounced increase of the functional and effective refractory period of the A V node (7)

Pharmacokinetic studies of (14C) verapamil in dogs indicate a short half life of 3 hours for the drug while its metabolites have a longer half life of 10 hours (9) Pharmacokinetic studies in man have not been reported so far

ECG studies by Bass and Friedemann (2) indicate, however that the drug must have a short biological half life of only three hours Our study confirms these findings as we have had to give the drug every third hour to maintain the effect of verapamil in some patients Usually we have administered the drug in the smallest amount which was effective to suppress the arrhythmia He

Fig 2 ECG in patient 20 showing atrial tachycardia with a rate 190 on admission One hour later A T with block 230 115 next morning sinus rhythm

Fig 1 Heart rate in patient 20 following treatment with verapamil

Fig 3 Heart rate in patient 21 following treatment with verapamil

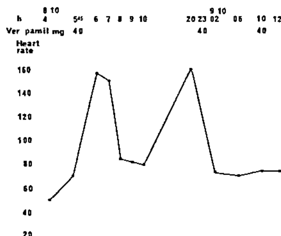


Fig 4 Heart rate in patient 13 following treatment with verapamil

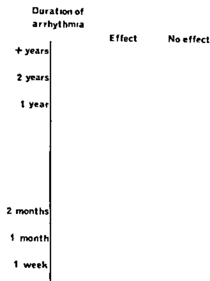


Fig 6 Relationship between duration of A T with block and effect of verapamil treatment

modynamic studies (1) have shown that verapamil has a slight negative inotropic effect It should therefore be given under careful observation to patients in congestive heart failure

We have not been able to find any other report on the effect of verapamil on A T with block Spurrel et al (14) studied the effect of verapamil on antegrade and retrograde conduction in the WPW syndrome They found only a minimal effect on the antegrade and retrograde conduction in the anomalous bypass in 8 patients with the WPW syndrome They found that supraventricular tachycardias could be terminated in these patients by verapamil and that the effect was due to slowing of conduction within the A V node They considered verapamil to be a safe drug in the treatment of the WPW syndrome as it does not shorten the refractory period of the bypass tract and that there is therefore a smaller risk of inducing ventricular fibrillation if atrial fibrillation should develop in these patients as is sometimes seen during digitalis treatment (16)

In our study verapamil was not however uniformly effective in the treatment of A T with block Sinus rhythm was obtained in 10 of 14 patients to whom it was given In 4 of these patients A T with block later recurred Continued administration of verapamil was however carried out in only 2 patients Experience in recent years of treatment with other antiarrhythmic drugs has shown the value of determining their serum concentrations as a guide to effective therapy It is therefore regrettable that it has so far not been possible to carry out pharmacodynamic and pharmacokinetic studies of this drug

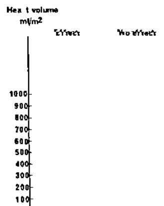


Fig 5 Relationship between heart size and effect of verapamil treatment

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A Controlled Clinical Trial of Treatment with ALG in Established Rejection of Renal Allografts

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ABSTRACT A controlled clinical investigation of the effect of equine anti human lymphocyte globulin (ALG) on established rejection after transplantation has been carried out in a group of 31 patients Compared with a control group the treated group showed a significantly positive effect of ALG in the form of a faster reversal of rejection episodes ($p<0.05$) Leucocytes, lymphocytes, erythrocytes thrombocytes and immunoglobulins were unaffected by the treatment, but there were signs of a complement-consuming process and an influence on blast transformation tests No rise in antibody titre against equine globulin was seen

The first basic investigations of anti lymphocyte serum (ALS) in animal experiments in 1961-67 (14 16 19 20 32 34) were followed in 1967 by the first trial of ALS in human kidney transplantation by Starzl et al (27) and then by other groups (8 29 33) Most of the published work is on treatment with anti lymphocyte globulin (ALG) given in the period immediately after transplantation ALG treatment of established rejection episodes has been studied only sporadically

This study was planned to show whether ALG treatment of established graft rejection gives a significantly faster reversal of rejection in a treated group compared with a control group which as far as possible differs from the treated group only with regard to ALG therapy

As nearly all rejections can be reversed by using conventional therapy with increased steroid doses it would not have been relevant to investigate whether ALG could increase the number of reversals in a group of patients The subject of this study however is theoretically interesting as it concerns the effect of ALG on rejection and clinically interesting since it may present the possibility of reducing the critical period in which extra corticosteroid therapy is necessary

METHODS AND MATERIAL

The investigation was planned as a controlled trial Patients were allocated randomly either to a treated group (+ALG) or to a control group (0 ALG)

The statistical method used was a sequential analysis (skew restricted sequential design) This method is patient saving and is the quickest way of reaching a result on predetermined levels of significance for type 1 and 2 errors As it was assumed that ALG treatment could not worsen the results of rejection therapy a one sided test was used The significance levels used in the construction of the sequential analysis diagram (Fig 1) were for errors of the first and second kinds $2\alpha=\beta=0.05$ and for the clinical significance $\theta=0.85$ Further details of the principles of this method can be found in publications by Bross (7) and Armitage (1)

In the sequential analysis method a significant result is only assured for one parameter and the analysis is finished when this result is obtained For other parameters alternative tests of significance must be used to detect differences in the measured values and in this study Mann Whitney s probability tests were employed The choice of main parameter is thus very important "Length of rejection defined as the period from diagnosis of *rejection to reversal was chosen here*

Twelve pairs of patients (details in Table I) came to be included in the sequential analysis When this analysis was concluded additional patients were still undergoing treatment so that 31 patients were included in the investigation as a whole (Table II)

All patients with a diagnosis of rejection made during the investigation period were included in the material except those who showed an anaphylactic reaction to the ALG preparation used in preliminary intracutaneous

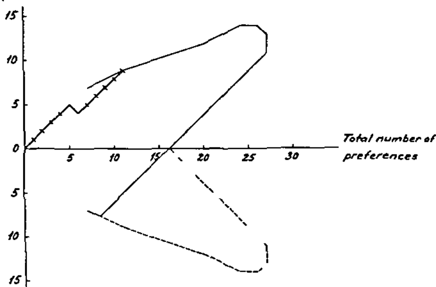


Fig 1 Sequential diagram for the controlled analysis

tests The only possibilities for withdrawal of a patient from the analysed material were death before reversal of rejection or a primarily incorrect diagnosis of rejection In both cases the withdrawn patients were replaced blindly by another patient in accordance with the principles of the analytical method

Diagnosis of rejection was made according to the common criteria decreasing creatinine clearance decreasing diuresis increase in leucocyte count fever hypertension etc In seven cases the diagnosis was made after supplementary graft biopsy and in a further eight cases it was confirmed by histological examination at a

Table I *Detailed particulars about the pairs of patients entering the sequential analysis*
RAT=renal allotransplantation CPN=chronic pyelonephritis RCD=renal cystic disease CGN=chronic glomerulonephritis MH=malignant hypertension

+ALG								0 ALG							
Pat no	Diagnosis	Age (y)	No of RAT	No of mismatches	No of rejections	Days from RAT to rejection	Days to reversal of rejection*	Pat no	Diagnosis	Age (y)	No of RAT	No of mismatches	No of rejections	Days from RAT to rejection	Days to reversal of rejection*
1	CPN	56	1	1	1	64	1	2	CGN	41	1	1	2	75	∞
4	RCD	51	1	2	1	24	4	3	CPN	56	1	1	2	123	∞
5	CPN	45	1	1	1	92	8	6	CGN	41	1	2	1	7	20
7	CPN	56	2	1	1	14	1	8	MH	49	1	2	1	27	2
13	CPN	50	1	2	1	19	16	9	CPN	45	1	1	2	138	∞
17	CGN	38	1	2	1	6	∞	11	RCD	51	1	2	2	146	2
18	CGN	28	1	1	1	7	3	12	RCD	41	2	2	1	7	4
19	CGN	38	2	1	1	149	1	14	RCD	36	1	2	1	7	∞
23	RCD	54	1	2	1	36	∞	15	CPN	50	1	2	2	45	∞
24	CGN	25	2	2	1	6	6	20	CGN	37	2	1	1	9	∞
25	CGN	19	1	2	1	12	3	21	CGN	38	2	2	2	190	4
29	CPN	42	1	2	1	39	8	26	CGN	26	1	2	1	5	∞

* Input data to sequential analysis

Table II *Late follow up of ALG treated and control patients*
RAT=renal allotransplantation

+ALG

Pat no	Trans plant ectomy	Exit	Graft survival[a] (d) From RAT	Graft survival[a] (d) From rejection	Patient survival[b] (d) From RAT	Patient survival[b] (d) From rejection
1		+	213	149	213	149
4			709	605	709	709
5		+	272	180	272	180
7		+	35	21	35	21
13			540	521	540	521
16		+	315	6	315	6
17	+	+	32	26	57	51
18	+		18	9	439	432
19			580	431	580	431
23	+		55	19	396	360
24			345	339	345	339
25			345	332	345	332
27		+	37	19	37	19
29			321	282	321	282

0 ALG

Pat no	Trans plant ectomy	Exit	Graft survival[a] (d) From RAT	Graft survival[a] (d) From rejection	Patient survival[b] (d) From RAT	Patient survival[b] (d) From rejection
2	+		409	334	432	357
3		+	213	90	213	90
6		+	73	66	73	66
8		+	114	87	114	87
9		+	607	469	607	469
11			709	563	709	563
12			583	576	583	576
14	+	+	11	4	68	64
15			540	495	540	495
20			427	418	427	418
21			580	390	580	390
22		+	21	10	21	10
26	+		7	2	337	337
28			466	286	466	286
30		+	180	136	180	136
31			288	264	288	264

[a] To transplantectomy death or Sept 30 1974 [b] To death or Sept 30 1974

later date Reversal of rejection was defined as occurring on the day after which a continual rise in creatinine clearance was seen

ALG and other immunosuppressive treatment

Equine ALG (Behringwerke) batch nos Op 501 and 502 was used The quality criteria for ALG were 1) skin allograft survival time>15 days (M speciosa) 2) lympho cytotoxic titre 1 1050–1 2500 3) rosette inhibition titre 1 16000–1 16000/32000 and 4) hemagglutination titre 1 32/64–1 16 Antibodies against erythrocytes thrombocytes plasma proteins and glomerular basement membrane antigens had been removed The resultant ALG preparation was an antispecies globulin with some anti-B cell activity and little anti T cell effect

The investigation was given the form of a fixed-dose trial using 20 mg ALG/kg until reversal of rejection and subsequently 10 mg/kg to day 21 after rejection (+ALG group) The ALG was dissolved in 250–500 ml 0 9% sodium chloride solution or 5% glucose solution and infused over 1–2 hours under observation As far as possible different veins were used from day to day

The remaining immunosuppressive treatment was the same in the two groups and consisted of prednisolone 1 g i v during transplantation followed by 50 mg/day for 5 days 1 mg/kg/day to day 30 then decreasing 2 5 mg/day per week to a maintenance dose of 10 mg/day Extra *prednisolone 30 mg/kg/day i v every second day* was given from diagnosis of rejection to reversal (maximum five times) Azathioprine was given in the maximal tolerable dose of 0–3 mg/kg/day depending on the leuco cyte count

Additional parameters

The patients were followed carefully from diagnosis of rejection to death or day 31 with a series of graft related parameters serum creatinine blood urea creatinine clearance diuresis urine protein WBC (leucocytes gran ulocytes eosinophil cells lymphocytes and monocytes) erythrocytes thrombocytes IgG IgA IgM IgD and IgE and complements C 3 and C 4 The results were processed in an IBM 360 computer (Fortran programme) For each patient the results of each parameter were plotted against time using an automatic plotter Approxi mately 400 curves were thus obtained for further evalua tion

RESULTS

Experimental technique

The experimental plan functioned well throughout the investigation period and no alterations were made In the preliminary intracutaneous test none of the patients showed any signs of anaphylactic reaction to equine globulin One patient was with drawn from the material because of an incorrect diagnosis which was rapidly invalidated by graft biopsy Two patients from the treated group and one from the control group were withdrawn from the analysis because of death before reversal of rejection

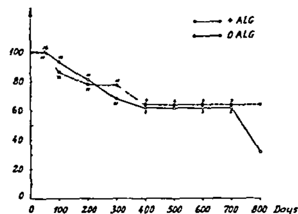


Fig 2 Cumulative patient survival from transplantation to death or Sept 30 1974 using the decrement method

Of the 12 pairs of patients necessary to reach a significant result in the sequential analysis one pair showed a negative effect of ALG treatment (Table I pair 17–11) one pair no difference (Table I pair 23–15) while all other pairs showed a positive effect The controlled analysis terminated with the following conclusion The trial was carried out according to the premises laid down in the plan and the results were plotted in the sequential analysis diagram (Fig 1) which was determined by the given values of α β and θ As the result line crosses the upper limit of the diagram it can be concluded that using the experimental technique described reversal of rejection was significantly faster in the treated group than in the control group with a 5% risk of type 1 error

Comparison of groups

A comparison between the +ALG group and the 0 ALG group (Table I) gave the following results The distributions of the primary kidney diseases were not significantly different There were no significant differences in age between the two groups (average age of +ALG group 41 8 years 0 ALG group 42 6 years) Neither was there any significant difference between the two groups with regard to the number of transplantations degree of matching number of rejections or timing of rejection in relation to transplantation A comparison of the amount of immunosuppressive treatment given in the two groups revealed no significant differences (Average doses per patient were as follows Prednisolone +ALG 48 2 mg/day 0 ALG 49 5 mg/day azathioprine +ALG 57 4 mg/day 0 ALG 69 6 mg/day solumedrol +ALG 8309 mg 0 ALG 7963 mg)

Long term observations of the groups

The results of long term observations of the patients are presented in Table II There were no significant differences between numbers of graftectomies or numbers of deaths in the two groups Surveys of survival times for grafts and for patients both in relation to time of transplantation and time of rejection did not reveal any significant differences Fig 2 shows the cumulative patient survival from transplantation to death or Sept 30 1974, using the decrement method

Additional parameters

As individual patients have differing immunocapacities at the start of rejection and because the curves obtained during the observation period showed large variations it was not possible to work

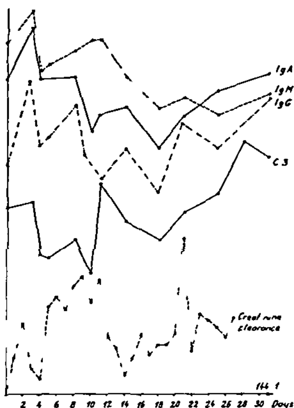


Fig 3 Change in immunoglobulins complement and creatinine clearance during rejection for one of the treated patients (no 1) The purpose is to show the relationships between the parameters and not their actual values For this reason units for the various parameters are not included

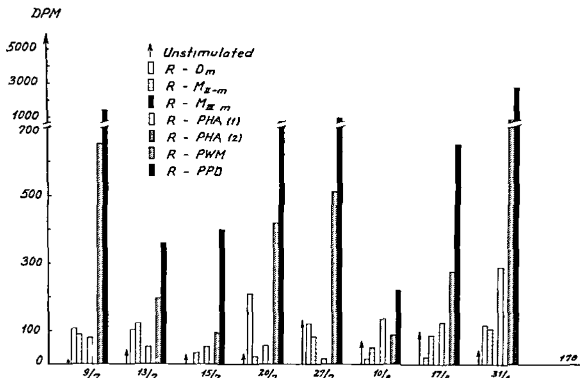


Fig 4 Blast transformation tests following the transplantation of patient 18 R=recipient D=donor m=mitomycin treated cells M_{II} and M_{III}=control cell populations of spleen cells *PHA* (*1*) and (*2*)=two different concentrations of phytohemagglutinin *PWM*=poke weed mitogen *PPD*=tuberculin purified protein derivative *DPM*=disintegrations/min

Rejection diagnosed 15/7 after initially good renal function reversed rejection 18/7 graftectomy 26/7 During the rejection crisis a lowered immune response of the peripheral lymphocytes was seen when the most immunocompetent cells were engaged in the graft rejection process In the period 15/5–26/7 when ALG was administered the renal function increased The immune response of the peripheral lymphocytes also increased but this increase was not directed against the graft

out an average for the progress of a group of patients The curves had to be considered individually and no true statistical evaluation was possible However some general tendencies were discerned

Kidney function Only creatinine clearance seemed to be suitable as a measure of graft function In a number of cases there was no correlation between creatinine clearance serum creatinine blood urea or diuresis In some cases serum creatinine and creatinine clearance varied counter actively for somedays and occasionally there were considerable changes in creatinine clearance several days before serum creatinine itself changed These are well known phenomena which seem to be emphasized in connection with graft rejection

White blood cell counts Leucocyte and lymphocyte counts were well correlated to graft function in only a few cases often no correlation was observed There was no relationship between leucocytes and their various subtypes except in cases of clear depression of the bone marrow Agranulocytosis after overdose of azathioprine was delayed so long after the overdose that daily leucocyte counts did not provide a prophylactic control in these cases (It is therefore important to keep to a maximum dose of 3 mg/kg/day) There were no characteristic differences between the two groups in any WBC in particular no differences in lymphocytes

Lymphocyte erythrocyte and thrombocyte counts For the evaluation of the effect of ALG on these parameters the following method was chosen The lowest values obtained in each parameter were taken as a measure of the effect of immuno suppressive treatment The coefficient of the slope between values at the start of rejection and on the day on which minimum values were obtained was taken a measure of the strength of the effect The

coefficients were plotted against the days of minimum value Using this method there appear to be no differences between the two groups and consequently there is no reason to relate the effect of ALG to a depletion of peripheral lymphocytes or to suspect ALG of containing antibodies against erythrocytes or thrombocytes

IgG IgA IgM IgD IgE complement C 3 and C 4 There were also large individual differences regarding these parameters The most characteristic difference between the treated group and the control group was in the values for C 3 and C 4 In the treated group there was a marked initial fall in nearly all cases while less than half the patients in the control group showed this fall IgG IgA and IgM were in most cases related to graft function while IgD and IgE remained in most cases completely unaltered at the same level during the rejection episode These relationships are illustrated in Fig 3

Glomerular basement membrane antibodies Graft biopsy material from a patient receiving ALG was examined using an immunofluorescence technique with conjugated anti horse globulin Only one patient was investigated in this way and there were no signs of deposition of equine ALG

Lymphocyte transformation tests The methods used and the results obtained during the course of transplantation and rejection have been described earlier (2 3) Fig 4 illustrates the progress of patient 18 who was treated with ALG with a successful reversal of rejection but afterwards was graftectomized for non immunological reasons

Serum titre for antibodies against equine globulin Passive hemagglutination sheep erythrocyte *agglutination and gel diffusion tests were used* There were no increases in titres for antibodies ainst equine globulin in the course of treatment These investigations are discussed further in another report (4)

DISCUSSION

This study was designed to give a rapid elucidation of the effect of ALG in the treatment of rejection It was planned largely along clinically oriented lines so that the results would be of practical relevance for wards which treat transplantation patients The following three factors are of importance in this connection

1) In order to keep the investigation clinically relevant only patients who could tolerate ALG would be included This was ascertained with an intracutaneous test before allocation of patients to the two groups This avoided the need to withdraw patients because of anaphylactic reactions which would otherwise only occur in the treated group and bias the investigation

2) Patients developing anaphylaxis during treatment would not be withdrawn from the analysed material even though the treatment were stopped otherwise the material would only contain patients who could tolerate the planned treatment carried through to its conclusion This could provide the basis for a positive result which might only be relevant for a small percentage of patients and would thus be clinically irrelevant

In practice none of the patients in the analysis fell into these two categories

3) All patients with acute rejection were included in the material without regard to degree of matching number of rejections the immunosuppressive treatment given before rejection or number of transplantations In this way the result could predict the effect of treatment in precisely the situation which is found in practice where a treatment is required which reverses rejection as quickly as possible without regard to its background

A review of the results revealed that in fact the two groups did not differ in respect of these factors

It was not considered necessary or possible in practice to carry out a double or triple blind investigation For similar reasons placebo treatment was not included in this study The possibility of an excessive diagnosis of rejection does not bias the analysis since all the patients in the material *were first subjected to randomization Any deficient* diagnosis will always be discovered corrected and included in the material before randomization As the point of time at which reversal of rejection occurs is defined in terms of a clearance measurement the result of the investigation is not influenced by subjective evaluations

The results indicate that the ALG used does not function through a peripheral lymphocytopenia but possibly by means of a complement-consuming process The investigation also showed that none of the measured values constitute a specially relevant measure for treatment and they can therefore be excluded from routine However WBC and thrombocyte counts as measures of bone marrow depression caused by immunosuppression and antibody

titres as a measure of anaphylactic reaction are included in the routine

Many animal-experimental investigations of the immunosuppressive effects of ALG have been carried out the majority showing a positive effect Human clinical investigations are more difficult to interpret partly because the variations in immunocapacity of patients and a number of immunological and non immunological factors relating to the graft complicate the situation and partly because of the difficulties associated with a clinically controlled investigation of the effects of immunosuppressive agents Because no one yet has ventured the use of ALG alone as immunosuppressive treatment investigations have always been based on the use of a combined therapy with ALG steroid and additional agents usually azathioprine

Since Starzl et al published the first human material (27) a series of reports have appeared (5 6 9 11 12 13 17 18 21 23 24 25 26 28 29 30 31) Only a few concern the use of ALG in the treatment of established rejection (11 12 18) the others dealing with ALG treatment in the period immediately following transplantation Most of these reports are of uncontrolled investigations and only a few can be mentioned as controlled clinical trials (5 13 22 23 24 30) Of these only one (30) and partly another (13) fulfil the rigorous requirements of a controlled investigation Most of the numerous reports conclude that ALG has a positive effect In an extended trial (31) presented at the Transplantation Congress in Jerusalem 1974 the conclusion was that immune responsiveness overrides the influence of available immunosuppressants including anti thymocyte globulin (ATG) Benefit from ATG was suggested only in selected groups

The present study is as far as can be ascertained the only controlled investigation of ALG treatment of established rejection Rigorous controls of randomization were used and the investigation was carried out as a fixed-dose trial With the statistical method employed here ALG was found to have a positive effect on established rejection Although it was not possible in the same material to demonstrate any significantly increased patient or graft survival such a result cannot be excluded but may be due to the limitations of the analytical method used On the other hand it may be that during ALG treatment of established rejection with the object of increased survival a reduced steroid dose should be used for it is commonly presumed that when the necessity of high-dose steroid is reduced so is the fatality of infections

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Uriglox® and Quantitative Urine Microscopy in Diagnosis of Urinary Tract Infection

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ABSTRACT The aim of this study was to find an alternative to the sole use of abundant cultural findings as a basis for the diagnosis of urinary tract infection (UTI) For this purpose the results obtained from the bacteriological culture of daytime urine specimens from 154 students by the dip slide method were checked against the findings from the quantitative culture microscopy and Uriglox® testing of the first morning urines voided later at home As a diagnostic criterion, the finding of 10 or more bacteria/ml urine in two successive cultures had an error of 19% For the simultaneous occurrence in the morning urine of abundant bacteria (≥10 /ml) and a subnormal glucose concentration (as revealed by the Uriglox test), this error was 1 5% Only the latter combination showed, therefore, the presence of UTI at the confidence level of ≥95% or was 'clinically significant ' The specificity indices for the Uriglox test and the quantitative culture were 0 99 and 0 97, respectively Microscopy of the morning urine showed 10^3 or more bacteria/ml in all the subjects with infection but the number of leucocytes was normal in a fifth of them The specificity indices for microscopic counts of 10^3 or more organisms/ml and 10 or more leucocytes/mm³ were 0 74 and 0 94 respectively For higher counts, i e 10^5 or more bacteria/ml and 50 or more leucocytes/mm³, the specificity index of positive microscopy was 1 0 This specificity level was, however, attained at the expense of the sensitivity, which for 10^5 or more organisms/ml was 0 67 and for 50 or more leucocytes/mm³ 0 53 It is concluded that abundant bacterial contamination of specimens often decisively complicates the diagnostic use of urine culture and therefore the combined use of quantitative culture, microscopy and the Uriglox test is recommended as the principal tool for the diagnosis of UTI in ordinary hospital and ambulatory health services

If urinary tract infections (UTI) are to be reliably diagnosed on the basis of consecutive cultural findings the frequency of abundant bacterial contamination (≥10^5 organisms/ml) of the urine specimens must be measured (5 19) Because this is difficult—if not impossible—to achieve in routine laboratory work the quantitative cultural diagnostics of UTI the Kass method (17 19) has had to be simplified for use in the ordinary health service (2) These simplified methods easily give rise to large numbers of false positive findings

For this reason we decided to study the usefulness both of microscopic counting of leucocytes and bacteria (9 24) and of the demonstration of subnormal glucose concentration by the Uriglox® test (25) as complementary urine examinations in the cultural diagnostics of UTI

MATERIAL AND LABORATORY METHODS

As part of a health screening of 545 female students the urine samples collected between 4 and 8 p m were examined by a *dip slide method of urinary culture* (Uricult® Orion) Within 1–5 weeks (average 2) the first morning urines voided at home were examined from 154 of them The morning specimens were studied by the dip slide and loop count methods of quantitative culture by the Uriglox® test (Kabi Stockholm) and by microscopy of the urine for leucocytes and bacteria

In the health screening two nursing orderlies specially trained for the purpose collected the midstream urine specimens after careful washing and drying of the vulva and inoculated the dip slides The dip slides were incubated in the Central Hospital Laboratory and read by the bacteriologist on duty (K O H or P G) For the collection of the morning specimens the students were furnished

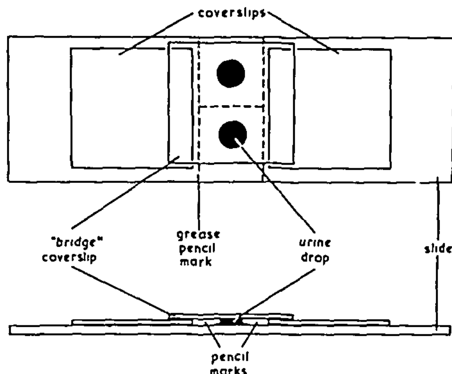


Fig 1 Disposable counting chamber used for urine microscopy (14)

with the following a wide mouthed sterile plastic container into which to void the urine a jar containing Uriglox preservative (Kabi Stockholm) a glass tube into which one drop of formalin was added and a list of detailed instructions According to the instructions food and drink were forbidden after 10 p m The first morning urine was collected as a clean-catch midstream sample directly into the wide mouthed container The Kabi Uriglox jar and the formalin tube were then to be filled and all three containers to be brought without delay to the Microbiological Laboratory of the Central Hospital Those samples not mixed with preservatives were inoculated onto dip slides and using a loop measuring 0 001 ml onto purple serum agar plates From the well shaken formalin tube a drop of urine was placed into a disposable counting chamber made of coverglasses and calibrated with red cell suspension as described by Hilson (14) (Fig 1) For determining the cell and bacterial contents both 10× and 40× objectives were used The jar containing the Kabi preservative was used for the Uriglox test according to the instructions given by the manufacturer

Diagnostic criteria

Quantitative culture was considered to be positive when the bacterial count was $\geq 10^5$/ml Microscopy was regarded as positive for bacteria when the count exceeded 10^3 organisms/ml and for leucocytes when it exceeded 10 cells/mm³ The Uriglox test was graded as positive only if the strip remained completely colourless

Since the bacterial contamination of a urine specimen causes both cultural and microscopic positivity these do not qualify as independent observations in the search for clinically significant combinations The opposite however is true for the simultaneous positivity of the Uriglox test and a bacteriological examination for which no common causes of false positives are known As the sole condition for the application of Kass statistical treatment to two sets of observations (19) is the mutual independence of false positive findings in these sets it gives a more reliable estimate for the significance level of the *simultaneous* occurrence of subnormal urinary glucose (positive Uriglox test) and high bacterial counts ($\geq 10^5$ organisms/ml) than that of two or more consecutive high counts which in some subjects is due to the tendency of the specimen to be contaminated continuously The various combinations of findings available in the study as diagnostic criteria are presented in Table I

Table I *Six different combinations of positive cultural and Uriglox® findings*

From the combinations of two tests only the framed one has diagnostic significance at the 95% level

	Findings in each combination*						Total positives in each examination
	I	II	III	IV	V	VI	
No of cases	14	1	3	13	2	1	
1st culture	+	−	+	+	−	−	30
2nd culture	+	+	+	−	+	−	20
Uriglox test	+	+	−	−	−	+	16

Estimated proportion of false positives in the findings of 1st culture 16/30=53% 2nd culture 5/20=25% Uriglox test 1/16=6%

* + =Positive culture ($\geq 10^5$ bacteria/ml urine) positive Uriglox test (<2 mg glucose/100 ml urine) − =negative

Table II *Quantitative findings in two successive bacterial cultures in the cases of infection detected and in the other students studied*

	Number of cases showing				
	≥10^5 bacteria/ml in both cultures	≥10^5 bacteria/ml in one of the two cultures	≥10^3–<10^5 bacteria/ml in one or both cultures	Negative results (<10^3 bacteria/ml in both cultures)	Total no of cases
Infected	14	1	0	0	15
Uninfected	3	15	69	52	139

Indices of diagnostic usefulness for all positive findings α 87/139 0 63 specificity $(1-\alpha)=0$ 37 $\beta=0/15$ 0 sensitivity $(1-\beta)=1$ 00 The corresponding indices for findings of ≥10^5 bacteria/ml α 18/139 0 13 specificity $(1-\alpha)=0$ 77 β 0/15 0 sensitivity $(1-\beta)$ 1 00 (The sensitivity indices are probably too high because the possible bacteriostatic property of the urine was not excluded and the diagnostic criterion ensured in the first place the presence of the infection) α=Proportion of positive findings in uninfected cases β=Proportion of negative findings in those with significant evidence of infection

RESULTS

Growths corresponding to 10 or more bacteria/ml urine were found in the first cultures (dip slides) of 30 students (Table I) In 13 of them the second culture from the morning specimen failed to confirm this result Consequently 13/30 (43%) of high counts found in the first culture were probably due to bacterial contamination Using this estimate (C_1) to calculate the error of diagnosis based on two consecutive high counts from the formula by Cohen and Kass (5) $C_2=100\ (C_1)^2$ we obtain $C_2=100\times(13/30)=19\%$ As this error is almost four times the 5% required for clinical significance as defined by Kass consecutive high counts could not in this study be used as the criterion of true bacteriuria The somewhat lower contamination rate of the second culture does not substantially affect this and cannot be properly used in the original Kass method based on two consecutive cultures

The Uriglox® reaction from morning urine indicated the presence of UTI in 16 of the students In 15 of them the culture of the same urine yielded 10^5 or more bacteria/ml Hence the error of the positive Uriglox finding (C) seemed to be somewhere around 1/16 or 6% Of the 20 high bacterial counts found in the second culture five were probably false positives three because of the 19% contamination rate of the 17 cases positive in both cultures (see above) and two because of the absence in these cases of any other evidence of infection The resulting estimate of the error in the second culture (C_1) 25% equals that obtained if all bacterial findings not confirmed by Uriglox are considered as contaminations The errors (C_{ux} and C_1) calculated in this way for the Uriglox test and the second culture are analogous to the error of the first culture (C_1) in the formula of Cohen and Kass Substituting the product of C_{ux} and C_1 for the power expression on the right hand side of the formula—this time the single test errors were calculated for two different kinds of examination—we obtain $100\times1/16\times5/20-1$ 5% for the error of the UTI diagnosis ($C_{u\ +cu}$) based on the combination of Uriglox and culture positivity This figure already suggests clinical significance Owing to the small number of cases however it does not differ significantly from 5% Therefore we inserted into the formula also the higher 95% confidence limit (6) of the error calculated for the Uriglox test from the 187 positive findings presented in Table V The estimate of C_{ux+cu} thus obtained 4 5% shows that this combination of findings indicates the presence of UTI at the 95% significance level if the proportion of high counts due to the contamination of culture specimens does not reach 30%

The following additional considerations and findings clarify or reinforce these statistical conclusions The three normal Uriglox findings obtained from 17 students with high counts in both cultures cannot be regarded as false negatives because this precise number represents the proportion of the students in whom both counts were probably due to specimen contamination None of the 18 subjects producing high bacterial counts with normal Uriglox results showed definite pyuria (>50 leucocytes/

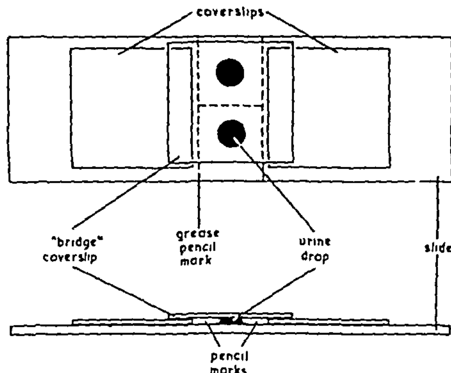


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Table V *The rates of false positives reported to date for the Uriglox® test in studies based on reliable diagnostic criteria*

Study	Criterion of diagnosis	Type and no of persons studied		Total no of positives	False positives (No)	False positives (%)
Fritz et al (11)	Recurrent high bacterial counts	Children	1 459	16	2	12
		Adults	2 452	90	11	12
Emmerson (8)	Recurrent high bacterial counts	Pregnant women	898	49	5	10
Paterson and Miller (21)	Bacteria in urine aspirated from bladder	Pregnant women	351	32	5	16
Total				187	23	12±5*

* 95% confidence limits (6)

Because the infection rate discovered in this study about 3% falls well within the range generally reported for similar female populations it seems safe to argue that all diagnostic tests with a specificity of 0 97 or less produce misleading results at least as often as correct positives in the screening of young women for UTI If all increased levels of urinary bacteria and cells are classified as positive findings both the quantitative culture and the microscopical examination of urine for bacteria and leucocytes must thus be considered to be unsatisfactory tests Interpreted in this way these tests produced false positive findings at the rates of 51–41 17 and 4% respectively (Tables II III and IV) Raising the lower limit of a positive finding to 10^5 bacterial/ml does not reduce the sensitivity of culture as an indicator of UTI but nor does it improve its specificity beyond 0 97 Bacterial counts above this limit were in fact recorded among the subjects not presenting significant evidence of UTI in 8% in the first and in 3% in the second culture For higher microscopical counts of bacteria ($\geq 10^5$/ml) and cells (≥ 50/mm^3) on the other hand the specificity indices were of an order (0 99 and 1 00) which no longer meant so much overdiagnosis Used within these limits the microscopical examination of urine produced only one positive finding ($>10^5$ bacteria/ml) outside the subjects with proven infections—as was the case with the Uriglox® test In microscopy however this specificity was achieved at the expense of sensitivity The latter was 0 67 for high bacterial ($\geq 10^5$/ml) and 0 53 for high leucocyte (≥ 50/mm^3) counts (Tables III and IV)

The formalized batches of all the morning specimens yielding on culture a count of $\geq 10^5$ bacteria/ml were microscopically positive for bacteria None of those high cultural counts was therefore solely the result of bacterial growth in the urine during transportation

DISCUSSION

Urine culture is indispensible for the identification and sensitivity testing of bacteria causing infections As a diagnostic examination however urine culture is one of the most difficult owing to the frequent occurrence of false positives caused by bacterial contamination of the specimens

The introduction of quantitative methods provided no definitive solution to this problem since a considerable proportion of even high bacterial counts ($\geq 10^5$/ml) is always due to specimen contamination no matter how carefully the specimens are taken

On the contrary interpretation of the quantitative results without adequate consideration of this fact has in practice led to uncontrolled overdiagnosis The use of two consecutive high counts as the diagnostic criterion does not correct this since the proportion of high counts due to contamination does not in routine laboratory work usually remain under the 20% level required for the proper use of this criterion It may also be asked whether the 95% diagnostic accuracy *implied by* clinical significance is at all sufficient for such a common disease as UTI For example applied to the 20 000 Finns presently being treated as chronic pyelonephritics at the expense of the community (28) this level of diagnostic accuracy would mean that at present 1 000 of them are taking drugs unnecessarily

Judging by our experience of present diagnostic practice the results of this investigation indicate replacement of the sole use of successive cultural findings by a thorough examination of the first morning urine as the principal diagnostic tool for UTI As components of this examination we propose quantitative microscopy the Uriglox® test and when necessary quantitative culture The usefulness of this combination lies essentially in the properties of the Uriglox test Firstly it is clearly more effective than repeated culturing for confirming the possible clinical significance of a positive bacterial finding The proportion of false results among the positive Uriglox findings has in all properly planned studies reported to date remained well below 20% (Table V) i e at levels not guaranteed for the culture of clean catch specimens even by the most successful aseptic techniques Secondly the sensitivity of the Uriglox test is not much lower than that of high bacterial counts Emmerson (8) found both 10^5 or more bacteria/ml urine and Uriglox positivity in all his 49 UTI cases Admittedly Fritz et al (11) as well as Paterson and Miller (21) recorded from their UTI patients numbering 97 and 31 respectively four cases of inexplicable Uriglox negativity each However recurrent heavy bacterial contamination of the specimen in one of 1 000 women examined by the former and the failure of asepsis in one of 100 bladder aspirations made by the latter could already have led to false UTI diagnosis in these eight cases For this reason the contradictory findings can just as well be attributed to the error of the diagnostic criteria as to the lack of sensitivity of the Uriglox test

Reports on the high incidence of Uriglox negative infections (23 29 30) anyway completely overlook the problem of specimen contamination Patients showing positive urine tests for diabetes or having an uncommon unfermentative urinary pathogen like pseudomonas give normal reaction with Uriglox strip despite the presence of 10^5 or more bacteria in *the bladder urine In these and other cases where* the requirements for applying the Uriglox test are not met the microscopical examination of urine often provides the additional evidence needed for the reliable diagnosis of UTI

The chief practical importance of urine microscopy lies in the fact that the demonstration of abundant bacteria and leucocytes in freshly voided clean-catch specimens enables immediate detection of many symptomatic bacteriurics Microscopic fields containing squamous cells and vaginal or periurethral bacterial flora must however be familiar to the microscopist Such findings are not to be classified as indicative of infection although they often contain many leucocytes and urinary pathogens In the diagnostic procedure we propose only the microscopy of uncentrifuged urine would be regularly used to detect UTI from daytime specimens The examination of urinary sediment would then be used only for qualitative demonstration of red cells casts or other unusual particles However if direct microscopy of a urine droplet—or its Gram stained smear (27)—is to be at all useful adequate attention should be given to the detailed technique sources of error and correct interpretation of the results A formalized sample of urine fixing its cell and bacterial contents at the immediate postvoiding level makes it possible for all bacteriological laboratories to keep in touch with the problems of urine microscopy and to offer the clinical laboratories the support they so often need to ascertain the reliability of their microscopical findings

Considered together negative microscopy of uncentrifuged urine and normal Uriglox finding will with reasonable accuracy ensure the absence of UTI in the ordinary medical examination To achieve this both examinations must however be applied to morning urine retained in the bladder over six hours and preferably with a pH above 5 6

In ambulatory care the use of such urines in this way and for quantitative culture is practicable only if the patients themselves collect the specimens on awakening In our study the culture specimens voided at home were contaminated less frequently than those taken at the doctor s office Successful applications of the Uriglox test in Sweden (4 25) have shown that the majority of female subjects can be relied upon to supply the communal health centre with a morning specimen taken according to the written instructions following an overnight fast *Such timing of the specimen also gives intraluminal* bacteria the growth period needed for high counts and even the possibility of adjusting the pH of urine to the level suitable for such a growth A dose of sodium bicarbonate (10 g for adults) the preceding evening ensures for the morning urine a pH above 5 6 which at least in vitro is the lower limit for rapid growth of most E coli strains (3) Unless the bacteria multiply for two hours within the blad

der—for which a 4 hour starting phase is often needed—the quantitative culture microscopy and the Uriglox test all give normal or equivocal results despite the presence of bacteriuria (18 22)

In ambulatory care such false negatives can be avoided only if the women are instructed in the collecting of morning urines and provided with the necessary equipment As shown in this study these measures also help to control the present over diagnosis of UTI *In our opinion the current* shortcomings already call for the implementation of such a practice Although the thorough examination of a single morning specimen on the lines suggested here usually leads to a definitive diagnosis in problem cases urine aspirated aseptically from the bladder or repeated culturing of the clean catch specimens are still necessary In most instances the former gives better results

ADDENDUM

After demonstrating in further studies that nitrite and Uriglox® tests are complementary to each other in disclosing the consumption of nutrients by the intraluminal growth of bacteria we have now added the nitrite test to the examinations made of the first morning urines Although there are many Uriglox positive infections in which the nitrite test remains negative in some dysuric UTI patients the opposite is true

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Differential Count of Urinary Leucocytes and Renal Epithelial Cells by Phase Contrast Microscopy

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ABSTRACT A total of 291 urine sediments from 255 patients with various renal or urinary tract diseases have been studied by phase contrast microscopy Based upon morphological criteria leucocytes were distinguished from renal epithelial cells and the white blood cells were classified either as mononuclear or polynuclear in 179 patients The percentage of the different cell types varied considerably between and within the different diseases The median values for polynuclear granulocytes were higher than 90% in bacterial renal or urinary tract disease and in poly cystic kidney disease In interstitial nephritis, nephrosclerosis and in renal transplanted patients the percentage of polynuclear granulocytes was somewhat lower, 76–85% In diabetes, amyloidosis, tubular nephrosis (necrosis) glomerulonephritis, lupus nephritis and endemic benign nephropathy there were 14–66% polynuclear granulocytes 29–33% mononuclear leucocytes were found in lupus nephritis and endemic benign nephropathy The greatest proportion of renal epithelial cells was found in endemic benign nephropathy, namely 49% 36% renal epithelial cells were found in tubular nephrosis (necrosis) and in glomerulonephritis The technique is rapid and inexpensive It facilitates differential diagnostics of urinary tract disease with pyuria

In unstained urine sediment it is often difficult to differentiate between the various types of white blood cells (WBC) by means of conventional light microscopy and to separate WBC and small renal epithelial cells from each other (1 9) With the aid of a cytocentrifuge and staining of the cells in the urine it is possible to identify eosinophil leucocytes (6) and even in some cases lymphocytes (7 9 12) Granulocytes and epithelial cells have been differentiated with peroxidase staining (1 8)

We have however had difficulty in differentiating between mononuclear WBC and small renal epithelial cells with staining technique We have therefore sought other simple technical methods The use of phase contrast microscopy (16) increases the possibility of differentiating between different cell types in urine (2 3 5 11 13) Using phase contrast microscopy we have attempted to differentiate urine cells obtained from patients with different renal or urinary tract diseases (10)

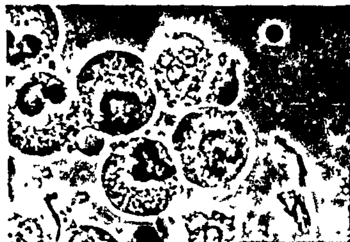
MATERIAL AND METHODS

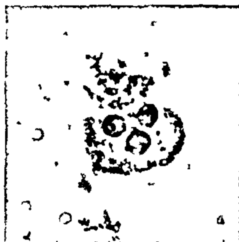
The risk of cells being obscured by salts or mucous or degenerating increases if the urine concentration is high and if the urine is old (4) Patients were therefore requested to empty the bladder and immediately afterwards consume 2 glasses of water in order to reduce the urine concentration They passed a 2 glass urine sample 1–2 hours later the second portion being used for examination

In about 25% of the cases the urine sample was obtained by percutaneous bladder puncture after the same preparations as above The 2 glass sample collection or bladder puncture were applied in order to reduce or avoid respectively contamination by cells from the lower urinary tract

The urine samples were centrifuged for 10 min at 2 500–3 000 r p m To retain as high a cell concentration as possible all the supernatant was carefully poured off the sediment was vigorously shaken and a small drop was placed on a glass slide using a thin plastic tube Placing a coverglass over the drop resulted in a thin liquid film which was necessary for good optical resolution Investigation of the preparation was performed within 1 hour after sampling as the number of degenerated cells can increase rapidly in alkaline urine (4) The slides were studied in a Zeiss phase contrast microscope usually at 400× magnification and at 1 000× using oil immersion for cell differentiation

The microscopic examinations were performed by two persons In most instances the investigator knew the patient s diagnosis Between 50 and 100 randomly selected cells were counted but excluding transitional epithelium





2



3



4



5 6

Photographs were taken with a phase contrast microscope at a magnification of ×1 000

Fig 1 Polymorphonuclear granulocytes one of which is degenerated and contains dense white nuclear segments
Fig 2 Polymorphonuclear granulocyte which has burst
Fig 3 Polymorphonuclear granulocytes surrounding a mononuclear leucocyte The nucleus of the mononuclear cell is indicated by an arrow
Fig 4 Two mononuclear leucocytes and between them a degenerated cell of uncertain origin
Fig 5 Two renal epithelial cells
Fig 6 Two renal epithelial cells

Table I *Urinary cell types percentage distribution in different diseases (median values)*

Diagnosis	No of pats	Polynuclear granulocytes	Mononuclear leucocytes	Renal epithelial cells
Cystitis	8	97	0 5	1 5
Pyelonephritis	18	96	2	2
Ren transplantatus cum bacteriuria	13	94	2	2
Renes cystici	10	91	5	4
Nephritis interstitialis	18	85	2 5	6
Nephrosclerosis	8	82	9	9 5
Ren transplantatus	17	76	6	14
Diabetes mellitus	5	66	9	25
Nephrosis (necrosis) tubularis	8	60 5	18	36 5
Amyloidosis renis	7	60	12	24
Glomerulonephritis	9	54	12	36
Lupus erythematosus disseminatus	10	51 5	29	19
Nephropathia endemica benigna	25	14	33	49

and RBC The cells were divided into three groups poly nuclear granulocytes mononuclear WBC and renal epithelium

As *polynuclear granulocytes* (Figs 1 and 2) were defined cells with two or more dark coupled nuclei and bright cytoplasm with highly motile granules In some cases however the granular movement had ceased and the nuclei lightened

As *mononuclear leucocytes* (Figs 3 and 4) were classified cells of the same size as the polynuclear granulocytes with rounded or kidney shaped dark nuclei and in general a light granular cytoplasm At first we attempted to distinguish between histiocytes lymphocytes and plasma cells but a clear distinction was not possible in many cases

Renal epithelial cells (Figs 5 and 6) were defined as cells with rounded excentrically located light-gray nuclei in which the nucleoli were clearly visible No motile granules were found in the cytoplasm Renal epithelial cells were generally slightly larger than polynuclear leucocytes but sometimes twice as large

Cells which were impossible to classify due to advanced degeneration occurred in most specimens Sometimes identification of cells was impossible even though they had not degenerated We could for example not decide with certainty whether cells containing large amounts of particulate material were macrophages or renal epithelial cells with inclusion bodies (15)

In all 291 sediments from 255 patients were checked 201 technically acceptable samples were obtained from 179 patients All but a few of the patients were admitted to the Clinic of Internal Medicine The diagnoses have been determined in most cases by renal angiography and renal biopsy All renal transplant patients and all except one of the lupus patients were treated with cytostatics and corticosteroids By interstitial nephritis we mean cases with a histological diagnosis of pyelonephritis upon renal biopsy but without known bacterial infection Endemic benign nephropathy (nephropathia epidemica) is an acute renal disease of probable viral origin occurring in Northern Scandinavia and Russia When more than one specimen from one and the same patient was examined the average percentage distribution for the cell types per patient was calculated

Statistical treatment of the material was carried out with Wilcoxon s rank sum test A significant level of $p<0\ 05$ was chosen All the differences between patient groups commented below were statistically significant at the chosen level

RESULTS

About two thirds of the specimens were appraisable There were several reasons why one third were unappraisable In most of these cases there were too few cells in the specimens In other cases appraisal was not possible because of cell degeneration All cases with more than 20% unclassified cells were excluded from the investigation

The median percentage values for the cell types in the different diseases are shown in Table I The individual values for polynuclear granulocytes are shown in Fig 7

However 23 patients belonged to disease groups consisting of one or two patients only or had two or more different renal diseases Therefore the results in these cases cannot be compared with others and they have been excluded from Table I and Fig 7 which contain the results from 156 patients

The median percentage of *polynuclear granulocytes* was highest in patients with bacterial renal or urinary tract disease and with polycystic renal disease In these patient groups 91–97% of the examined cells were granulocytes These values were significantly higher than those found in diabetes amyloidosis tubular nephrosis (necrosis) glomerulonephritis lupus nephritis or endemic benign nephropathy In interstitial nephritis nephro-

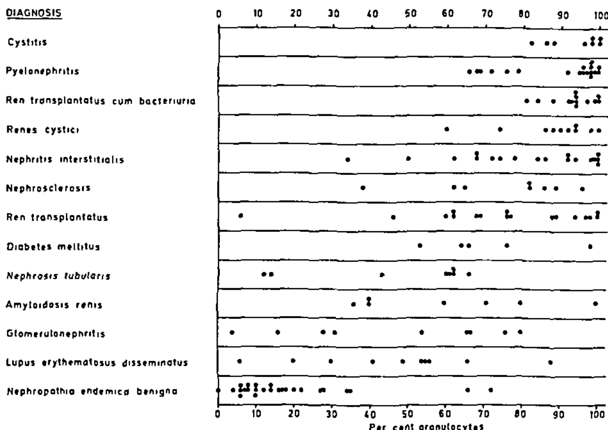


Fig 7 Percentage of granulocytes in 156 patients with different diagnoses

sclerosis and in renal transplanted patients 76–85% granulocytes were found These figures were significantly higher than those found in tubular nephrosis glomerulonephritis and lupus nephritis 51 5–60 5% granulocytes were found In endemic benign nephropathy 14% granulocytes were found which was the lowest percentage

Sternheimer and Malbin (14) who used stained sediments found that the polynuclear granulocytes had a distinctive appearance in pyelonephritis namely large lightly stained cells with lively motile granules In the present investigation cells corresponding to their description were observed in all the conditions investigated We had the impression however that the cells were larger in hypotonic than in more concentrated urine

The median percentage of *mononuclear leucocytes* was in general inversely proportional to the percentage of granulocytes In lupus nephritis and endemic nephropathy 29–33% mononuclear leucocytes were found In the bacterial diseases cystitis pyelonephritis and in renal transplanted patients with urinary tract infection and in polycystic renal disease only 0 5–5% of the cells were mononuclear leucocytes

The median percentage of *renal epithelial cells* was higher in endemic nephropathy (49%) lupus nephritis (19%) glomerulonephritis (36%) and tubular nephrosis (36 5%) than in bacterial renal or urinary tract disease and polycystic renal disease where 1 5–4% of the cells were considered to be of renal epithelial origin

DISCUSSION AND CONCLUSIONS

In almost all cases of significant bacteriuria more than 90% of the urinary cells were granulocytes An equally high percentage of granulocytes was also often found in cases without bacteriuria The great majority of these patients had a diagnosis of cystitis pyelonephritis polycystic kidney disease or had a transplanted kidney Bacterial infection is common in these conditions A high percentage of granulocytes indicates, but is not proof of bacterial urinary tract disease

A relatively high percentage of mononuclear

leucocytes was found in immunologic disease (lupus nephritis glomerulonephritis) and in acute kidney disease (tubular nephrosis endemic benign nephropathy) It is our experience that the finding of a high percentage of mononuclear leucocytes can give valuable information for the diagnosis

The percentage of renal epithelial cells was found to be highest in acute renal diseases such as tubular nephrosis (necrosis) and endemic benign nephropathy It was sometimes high also in immunologic disease diabetes and amyloidosis but in our opinion usually in the acute phase

Our greatest technical problem has been to concentrate the cells Another problem is the rapid degeneration of cells in urine and the concomitant difficulty of identifying the cells We have found phase contrast microscopy useful but not entirely satisfactory A comparative investigation between phase contrast microscopy of unstained sediment and light microscopy of fixed and stained specimens has been initiated

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Iron Absorption in Patients with Chronic Renal Failure not Requiring Dialytic Therapy

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ABSTRACT Gastrointestinal iron absorption, by means of whole body counting has been measured in 15 patients with chronic renal failure, not requiring dialysis Whole body retention 14 days after oral administration of 10 μCi ^{59}Fe together with a carrier dose of 10 mg Fe $^{+}$ was taken as expression of absorption The percentage incorporation in the total erythrocyte mass of administered ^{59}Fe (erythrocyte *incorporation) and absorbed ^{59}Fe (red cell utilization)* was estimated as well Iron absorption was 9 6±2 0 (S D) % (geometric mean) and erythrocyte incorporation 7 5±2 3 (S D) % (geometric mean) while red cell utilization averaged 80 3±4 8 (S E M) % (arithmetic mean) None of these parameters were significantly different from those obtained in a normal control group (p>0 5, p>0 9 and p>0 2 respectively) *The correlation between iron absorption* and erythrocyte incorporation was highly significant (r=1 00 p<0 001) Iron supplementation is often indicated in the investigated category of patients due to increased blood loss and insufficient iron intake and should be given orally in consideration of the normal gastrointestinal absorption

Anemia is an almost constant feature of chronic renal failure and is usually classified as being normochromic and normocytic (11) The etiology of this anemia seems complex Shortened erythrocyte life span (11 12 14) suppression of marrow function due to uremic toxins (11 13 18) and failing production of renal erythropoietic stimulating factor (REF) (13) may all contribute to the anemia to a varying extent Inadequate erythropoiesis caused by reduced marrow stimulation via REF is now considered to be the major pathogenic factor responsible for the development of anemia in chronic renal failure (11 13 30)

Furthermore one must recognize the existence of an often considerable iatrogenic component in the anemia due to blood sampling for routine check ups and research (22) In order to provide optimal conditions for marrow function under these circumstances it is important to avoid any treatable deficiency state regarding substances necessary for erythropoiesis and hence to ensure an adequate supply of proteins vitamins including B_1 and folate and iron Previous investigations concerning iron absorption in subjects with reduced renal function have yielded contradictory results (2 16 36 37) and for that reason the present study was undertaken in order to reexamine gastrointestinal iron absorption in patients with chronic renal failure

CASE MATERIAL

Fifteen patients (7 males 8 females) with chronic renal failure not requiring dialysis participated in the study Details of the patients are listed in Table I All had 24-hour endogenous creatinine clearance ≤12 ml/min and judged by this criterion the duration of the uremic condition varied from 1 to 25 month Kidney function was stable during the investigation and proteinuria ranged from 0 6 to 4 5 g/24 h (mean 1 8) None of the patients had been subjected to ventricular surgery or had infection at the time of the study 11 were taking a protein restricted diet containing an average of 0 7 g protein/kg b wt /day 4 had unrestricted protein intake All patients had negative Coombs test normal serum bilirubin no clinical malassimilation and no signs of protein deficiency judged by the serum protein and plasma albumin One patient (no 10) had repeated menorrhagias the others had no abnormal blood loss gastrointestinal bleeding being excluded by negative benzidine tests No iron therapy or blood transfusion had been administered in the year preceding the investigation

Table I *Clinical renal and hematological data on 15 patients with chronic renal failure investigated for iron absorption*

Pat no	Sex	Age (y)	Diagnosis	Serum creatinine (mmol/l)	Creatinine clearance (ml/min)	Serum urea (mmol/l)	Serum B_{12} (pmol/l)
1	♂	54	Polycystic kidneys unilateral nephrectomy	1 02	8 5	25	703
2	♂	42	Chronic pyelonephritis	0 56	9 7	19	755
3	♂	37	Chronic glomerulonephritis	0 71	10 2	26	618
4	♂	26	Gouty nephropathy unilateral renal aplasia	0 74	10 0	23	628
5	♂	51	Chronic glomerulonephritis	0 61	11 1	21	496
6	♂	19	Chronic pyelonephritis	0 67	12 0	28	407
7	♂	27	Chronic pyelonephritis	0 57	12 0	27	681
8	♀	31	Nephrosclerosis	0 34	12 0	18	646
9	♀	54	Polycystic kidneys	0 78	7 3	29	696
10	♀	35	Chronic pyelonephritis	0 57	8 3	36	1 206
11	♀	56	Polycystic kidneys	0 65	8 2	30	629
12	♀	51	Chronic pyelonephritis	0 52	7 9	22	644
13	♀	47	Chronic pyelonephritis	0 72	9 4	33	533
14	♀	54	Chronic pyelonephritis	0 66	7 3	13	481
15	♀	55	Chronic pyelonephritis	0 78	8 0	30	915
Arithmetic mean		43		0 66	9 5	25	669
S D		13		0 15	1 7	6	192
Normal range				0 04–0 13	96–120	3 3–7 5	140–600

METHODS

Iron absorption was measured by whole body counting using the method described in a previous paper (26)

All non vital medicine was withheld 4 days before the study and blood samples were avoided during the investigation period

After an overnight fast background activity was determined in each subject before the oral administration of 10 µCi $^{59}Fe^{2+}$ (as citrate) together with a carrier dose of 9 9 mg Fe^{2+} (as sulphate) Measurements of the whole body ^{59}Fe activity were performed at 4 hours and 14 days after administration of the isotope and corrected for background and radioactive decay before calculation of the percentage absorption of iron Erythrocyte volume was estimated according to the method described by Jarnum (23) and at the last counting procedure blood samples were drawn in order to assess the ^{59}Fe activity From these measurements were calculated the erythrocyte incorporation of ^{59}Fe (EIC) i e the percentage of administered ^{59}Fe recovered in the total erythrocyte mass and the red cell utilization i e the percentage of absorbed ^{59}Fe recovered in the erythrocytes Hematological parameters including Hb mean corpuscular volume (MCV) mean corpuscular Hb concentration (MCHC) hematocrit serum iron plasma transferrin and plasma total iron binding capacity (TIBC) were estimated by procedures referred to in our earlier paper (26) Reticulocyte counts were corrected for anemia according to Shaw and Scholes (35) Erythrocyte glucose-6-phosphate dehydrogenase was measured using the method of Kornberg and Horecker (25) serum vitamin B_{12} by the procedure of Karstoft (24) and erythrocyte folate with a microbiological assay Bone marrow specimens were obtained by iliac crest puncture stained for iron with Prussian blue whereafter hemosiderin iron content was graded according to Rath and Finch (34) Help and advice in the technical preparation and the assessment of the marrow aspirates were given by the Institute of Pathology Rigshospitalet

The control group consisted of 27 healthy subjects details concerning this group are published elsewhere (26)

The logarithms of the values for iron absorption and EIC were employed in the calculation of the geometric mean (the antilogarithm to the mean of the logarithmic values) according to Cook et al (8) In statistical analysis regression equations were calculated after the method of least squares and the Mann Whitney rank sum test was used to evaluate significance of differences between patients with chronic renal failure and control subjects

RESULTS

Clinical hematological and biochemical data together with results obtained for iron absorption and EIC are given in Tables I and II

Erythrocyte folate (nmol/l)	Erythrocyte glu cose-6-phosphate dehydrogenase (U/mean eryth rocyte)	Marrow ron (0–4)	Corrected ret culocyte count (1/1000)
589	?28		13
	189		
3 8	260	1	2
347	283	1	3
297	196	2	8
370	?12	1	18
295	?16	1	9
325	?05	1	1?
512	266	0–1	11
335	181	1	5
	209	1	5
	236	1	4
	234	1	18
400	215	1	5
525	306	1	4
393	228		8
10?	38		5
247–665	205–3 0		<1?

Iron absorpt on and er tl roc te
i corporat on of ^{59}*Fe*

In the pat ent group iron absorption ranged from 4 0 to 5? 7% with an arithmet c mean of 1? 4+1? 0 (S D)% (F g 1) and a geometric mean of 9 6+? 0 (S D) % EIC ranged from ? 2 to 5? 2% with a geometric mean of 7 5+? 3 (S D) % Arithmetic mean red cell ut lizat on of absorbed ^{59}Fe was 80 3+4 8 (S E M)%

In the control group arithmet c mean iron absorp tion was 11 5+10 4 (S D)% (F g 1) and geometric mean 8 5+? 1 (S D)% wh le geometr c mean EIC was 7 7+2 2 (S D)% and arithmet c mean red cell util zat on of absorbed ^{59}Fe 9? 9+4 0 (S E M) % (26)

There was no s gn ficant d fference between patients and controls concern ng iron absorption EIC and red cell util zat on ($p>0$ 5 $p>0$ 9 and $p>0$? respect vely)

The correlat on between ron absorpt on (x) and EIC ( ) was h ghly s gn ficant both n pat ents (y 1 0?1x 1 80 r 1 00 $p<0$ 001) and n controls (y 0 884x+0 48 r 0 96 $p<0$ 001) as sho vn in F g 2

Iron absorpt on showed no correlat on to serum ron TIBC transferrin saturat on and the degree or durat on of the uremic cond tion

He natolog cal st d es

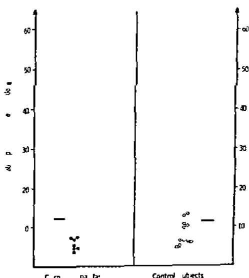
Five pat ents had Hb values within the normal range but in the ent re patient group the Hb level was sign ficantly lower ($p<0$ 004) than in the con trol group the anem a was normocyt c and normo chrom c The corrected ret culocyte count showed no sign ficant variat on between the two groups ($p>0$ 3)

Most of the pat ents demonstrated normoplastic marrow morphology with normoblastic erythro poiesis exceptions being one patient (no ?) with hypoplast c and one (no 1?) with hyperplastic marrow Stainable marrow iron was present in small quantit es in most pat ents and absent in one (Table I)

B ocl e cal st d es

Serum ron and plasma transferrin levels were lower in the pat ent group than in the control group ($p<0$ 01 and $p<0$ 03 respectively) whereas no d fference was found concern ng transferr n satura tion ($p>0$ 7)

Serum B $_{2}$ and erythrocyte folate values were normal and erythrocyte glucose 6-phosphate de hydrogenase was within the lower half of the nor mal range (Table I)



F g 1 Absorpt on of ^{59}Fe n pat ents w th chron c renal fa lure and con rols

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Clinical hematological and biochemical data together with results obtained for iron absorption and EIC are given in Tables I and II

Erythrocyte folate (nmol/l)	Erythrocyte glucose-6-phosphate dehydrogenase (U/mean erythrocyte)	Marrow iron (0–4)	Corrected reticulocyte count (1/1 000)
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512	266	0–1	11
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	209	1	5
	236	1	4
	234	1	18
400	215	1	5
525	306	1	4
393	228		8
102	38		5
247–665	205–3 0		<12

Iron absorption and erythrocyte incorporation of ⁵⁹Fe

In the patient group iron absorption ranged from 4 0 to 52 7% with an arithmetic mean of 12 4+12 0 (S D)% (Fig 1) and a geometric mean of 9 6+2 0 (S D) % EIC ranged from 2 2 to 52 2% with a geometric mean of 7 5+2 3 (S D) % Arithmetic mean red cell utilization of absorbed ⁵⁹Fe was 80 3+4 8 (S E M)%

In the control group arithmetic mean iron absorption was 11 5+10 4 (S D)% (Fig 1) and geometric mean 8 5+2 1 (S D)% while geometric mean EIC was 7 7+2 2 (S D)% and arithmetic mean red cell utilization of absorbed ⁵⁹Fe 92 9 4 0 (S E M) % (26)

There was no significant difference between patients and controls concerning iron absorption EIC and red cell utilization ($p>0.5$ $p>0.9$ and $p>0.2$ respectively)

The correlation between iron absorption (x) and EIC ( ) was highly significant both in patients (1 02x 1 80 r 1 00 $p<0.001$) and in controls (0 884x+0 48 r 0 96 $p<0.001$) as shown in Fig 2

Iron absorption showed no correlation to serum iron TIBC transferrin saturation and the degree or duration of the uremic condition

Hematological studies

Five patients had Hb values within the normal range but in the entire patient group the Hb level was significantly lower ($p<0.004$) than in the control group the anemia was normocytic and normochromic The corrected reticulocyte count showed no significant variation between the two groups ($p>0.3$)

Most of the patients demonstrated normoplastic marrow morphology with normoblastic erythropoiesis exceptions being one patient (no 2) with hypoplastic and one (no 12) with hyperplastic marrow Stainable marrow iron was present in small quantities in most patients and absent in one (Table I)

Biochemical studies

Serum iron and plasma transferrin levels were lower in the patient group than in the control group ($p<0.01$ and $p<0.03$ respectively) whereas no difference was found concerning transferrin saturation ($p>0.7$)

Serum B ₂ and erythrocyte folate values were normal and erythrocyte glucose 6-phosphate dehydrogenase was within the lower half of the normal range (Table I)

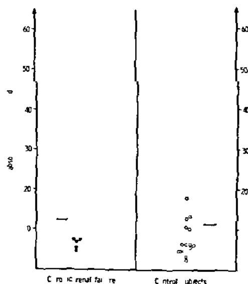


Fig 1 Absorption of ⁵⁹Fe in patients with chronic renal failure and controls

Table II *Hematological data iron absorption and erythrocyte incorporation of ^{59}Fe (EIC) in 15 patients with chronic renal failure*

Pat no	Hb (mmol/l)	Hematocrit	MCHC (mmol/l)	MCV (fl)	Serum iron (μmol/l)	Plasma transferrin (μmol/l)	Plasma TIBC (μmol/l)	Transferrin saturation (%)	^{59}Fe absorption (%)	EIC (%)
1	5 2	0 25	21 6	85	17 5	19 6	39 2	44 6	4 6	2 4
2	7 0	–	20 2	89	17 3	24 2	49 0	35 3	10 9	12 5
3	6 1	0 25	20 1	90	18 3	22 9	45 8	40 0	5 2	3 1
4	5 3	0 25	20 1	91	11 6	22 8	45 6	25 4	14 8	12 5
5	7 3	0 35	19 7	91	15 2	27 2	54 4	27 9	12 8	11 6
6	7 2	0 32	20 7	84	14 8	26 6	53 2	55 6	7 0	6 5
7	8 7	0 41	20 0	85	14 4	34 7	69 4	20 7	4 0	2 2
8	7 5	0 35	20 1	89	14 7	25 6	51 2	28 7	20 4	18 7
9	8 1	0 39	19 6	85	10 7	33 2	66 4	16 1	4 6	4 4
10	4 8	0 23	19 4	69	4 6	39 5	79 0	5 8	52 7	52 2
11	7 4	0 35	20 6	85	18 9	26 0	52 0	36 4	7 3	4 8
12	8 3	0 39	20 0	90	14 0	23 1	46 2	30 3	13 5	11 4
13	6 0	0 30	19 6	91	23 0	38 9	77 8	29 6	7 2	4 3
14	7 7	0 37	19 3	84	13 3	25 8	51 6	25 8	10 1	8 5
15	7 0	0 27	23 4	89	12 7	31 1	62 2	20 4	11 0	8 0
Arithmetic mean	6 9	0 32	20 3	87	14 7	28 1	56 2	29 5	12 4	
S D	1 2	0 06	1 0	6	4 2	6 0	12 1	12 1	12 0	
Geometric mean									9 6	7 5
S D									2 0	2 3
Normal range	7 0–10 5	0 35–0 52	18 6–22 3	81–109	10 7–34 0	24 2–47 7	48 4–95 4		1 9–38 3[a]	1 6–37 0[a]

[a] Larsen and Milman (26)

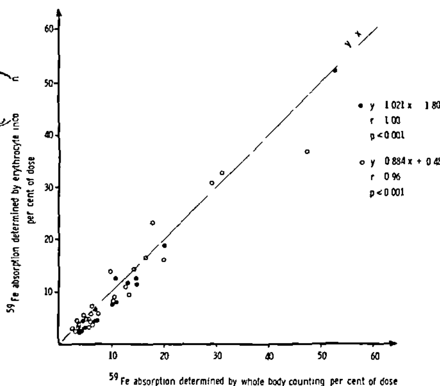


Fig 2 Relation between iron absorption and erythrocyte incorporation of ^{59}Fe in patients with chronic renal failure (●) and controls (○)

Table III *Iron absorption values from literature in patients with chronic renal failure not requiring dialytic therapy*

WBC=whole body counting

Authors	No of subj	Creatinine clearance (ml/min)		Method	Carrier dose	^{59}Fe absorption (% of dose)		
		Mean	Range			Arithmetic mean	Range	Significant difference from control subject
Takasugi & Imura (37)	6 ♂	24 6	10 4–37 0	Fecal recovery	5 mg Fe	37 8+9 4 (S E M)	9 8–78 0	No
Boddy et al (2)	4 ♂ 8 ♀	7 6	4–10	WBC	5 mg Fe^{3+} as chloride	3 5+0 9 (S E M)	0 1–10 3	Yes (lower)
Fam et al (16)	8 ♂ 3 ♀	Uninformed		WBC	74 mg Fe^{2} as sulphate	10 3	Uninformed	Yes (lower)
Sulin & Blumberg (36)	9 ♂ 6 ♀	≥24 8 subj <12 7 subj		WBC	4 mg Fe^{2+} as sulphate	21 5±16 4 (S D)	3 5–59 5	No
Present study	7 ♂ 8 ♀	9 5	7 3–12 0	WBC	10 mg Fe^{2+} as sulphate	12 4±12 0 (S D)	4 0–52 7	No

DISCUSSION

Previous investigations on iron absorption in chronic renal failure are summarized in Table III

Boddy et al (2) and Fam et al (16) found significantly lower iron absorption levels in uremic subjects than in normal controls and assumed that this was a consequence of decreased erythropoiesis and not caused by an abnormal intestinal handling of iron (2) On the other hand Takasugi and Imura (37) and Sulin and Blumberg (36) found iron absorption to be unaffected in patients with various degrees of renal insufficiency

From these investigations and the present study it appears that iron absorption values both in patients and in normal subjects are scattered over a wide range These variations may at least partly be explained by differences in erythropoietic activity and available iron stores (3 21)

It is generally accepted that small intestinal function is not appreciably affected in uremia although recent investigations have demonstrated minor changes in intestinal morphology and decreased dipeptidase and disaccharidase activity of small intestinal mucosa in some patients with chronic renal failure (10 29) None of the patients studied showed clinical signs of malassimilation and the iron absorption mechanism was evidently unaffected by the uremic condition

The EIC measurement was primarily intended as a control on iron absorption values and the correlation between these two parameters emphasizes the reliability of the whole body counting method in the assessment of iron absorption Fig 2) The application of the more inaccurate fecal recovery technique probably explains why Takasugi and Imura (37) failed to demonstrate such a correlation

Despite the correlation between iron absorption and EIC the latter parameter alone cannot be used as an expression of absorption as several factors may depress the EIC such as decreased erythropoiesis not accompanied by a proportionate reduction in iron absorption or the fact that part of the absorbed iron may be temporarily deposited in the liver before being used for erythropoiesis (17)

The red cell utilization of i v or orally administered ^{59}Fe is often subnormal in chronic renal failure (2 12 13 19 27 33 37) Concerning the intraindividual utilization of i v versus orally administered ^{59}Fe almost identical values have been found in patients undergoing regular dialysis (4) however this subject needs further investigation *The slightly lower EIC and red cell utilization* of absorbed ^{59}Fe and the absence of reticulocytosis in spite of anemia are indicative of a moderately depressed erythropoietic activity in the uremic patients

Hyperhemolysis is generally absent or slight in subjects with chronic renal failure (11 12 14) and

our patients did not demonstrate signs of severe hemolysis as indicated by the normal serum bili rubin and the low normal values of erythrocyte glucose 6-phosphate dehydrogenase which is a sensitive indicator of the erythrocyte age (38)

Bone marrow morphology was normal except in two patients These findings are in accordance with those reported by Callen and Limarzi (5) The continued presence of stainable marrow iron in case 10 despite a clear iron deficiency anemia is most likely explained by the slow and delayed mobiliza tion of iron from the hemosiderin deposits (15)

Plasma transferrin values were decreased in pa tients with chronic renal failure as earlier demon strated by Eklund et al (12) and Ooi et al (32)

The small quantity of stainable hemosiderin mar row iron present in most of the patients does not necessarily indicate reduced iron depots as the concentration of non heme marrow iron was not estimated (39) On the other hand several factors predispose to the development of slight iron defi ciency such as increased blood and urinary iron loss (6 9) low iron consumption and less favora ble dietary absorptive conditions The protein restricted diet contains approximately only 10 mg iron/day because the iron rich nutritives meat and blood products are omitted Hb iron is absorbed more readily than elemental iron (1 7) and meat facilitates iron absorption (28)

In the assessment of iron absorption under physiological circumstances it is important to rec ognize that the presence of food normally affects absorption in a negative direction (20) and that the iron absorption test is carried out during ideal absorptive conditions i e in the fasting subject

In conclusion the present study demonstrates that the iron absorption capacity is unimpaired in subjects with chronic renal failure However the increased blood loss the low dietary iron content and the unfavorable absorption milieu occasionally make iron supplement necessary Iron should be administered orally and the indication for par enteral iron therapy limited to patients with gas trointestinal disorders impairing iron absorption

ACKNOWLEDGEMENTS

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Waldenstrom s Disease with an IgA Paraprotein

Report of a Case

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ABSTRACT A patient is described, in whom the diagnosis of Waldenstrom s disease was made on the basis of clinical and morphological findings, but whose M-component belonged to the IgA class of immunoglobulins

The diagnoses of multiple myeloma and of Waldenstrom s macroglobulinaemia are based on clinical morphological radiological and biochemical data (14) In essence multiple myeloma is a malignant tumour of plasma cells which synthesize immunoglobulins or parts thereof In exceptional cases this immunoglobulin is of the IgM class (13) Waldenstrom s disease on the other hand runs a relatively benign course The patients complain of fatigue and on physical examination the major finding is a lymphadenopathy with a spleno and hepatomegaly Usually there is an anaemia and bleeding manifestations are often recorded Bone lesions are absent The bone marrow smear shows a preponderance of lymphocytic elements usually in combination with an increased number of plasma cells and mast cells In about 20% of the cases the disease is associated with the presence of non reticular neoplasms (11) Classically the typical so called lymphoid reticulum cells synthesize the characteristic monoclonal macroglobulin (3)

In this report a patient will be described in whom the diagnosis of Waldenstrom s disease was made but whose paraprotein belonged to the IgA class

CASE REPORT

A milkman 73 years old was referred to the Medical Outpatient Department (no 287/62) of the Leiden University Hospital in 1962 complaining of fatigue weakness and dyspnoea on exertion The past history revealed a carcinoma praeputii in 1960 and a basalioma and a melanoma of the skin in 1961 These had been removed without signs of recurrence The family history revealed no abnormalities On physical examination no abnormalities were found

Laboratory investigations showed an extremely high ESR (151 mm/1st hour) an anaemia (Hb 7 g/100 ml erythrocytes 3 mill /mm³) with reticulocytosis (5%) and a thrombocytopenia (70000/mm³) with a normal bleeding time Other routine blood examinations were normal

The total serum protein and the β globulin fraction were elevated (9 2 and 4 7 g/100 ml respectively) On agar and immunoelectrophoresis an IgA paraprotein of the κ type was found (Fig 1) The determinations of the immunoglobulin levels gave the following values IgG 38% IgM 45% IgA 3400% and IgD 0% (normal values 100%) On X ray examination there were no bone lesions

The bone marrow examination revealed a picture characteristic of Waldenstrom s disease (Fig 2) The number of lymphocytic elements was greatly increased with an excess of basophilic leucocytes The number of plasma cells was within the normal range (3%) and the other cell types were practically absent There was no Bence Jones proteinuria A diagnosis of Waldenstrom s disease with IgA paraprotein was made

Treatment and course (Fig 3) Initially several blood transfusions were given followed by a period of combined administration of an alkylating agent with prednisone This resulted in a subjective and an objective improvement The therapy was discontinued 1½ years later Two years later the patient was admitted because of extreme weakness and difficulty in walking due to a polyneuropathy The haematological picture was again as seen on the first admission

The combined therapy was reinstituted and plasmapheresis was performed several times A slight improvement was noted but after 6 months of cytostatic therapy this had to be discontinued because of bone marrow insufficiency Prednisone was stopped after one year because of gastric complaints During the first six months after cessation of all treatment no major problems were encountered He was admitted again in Nov 1968 because of epistaxis The blood picture showed a pancytopenia and the bleeding time was greatly prolonged The total serum

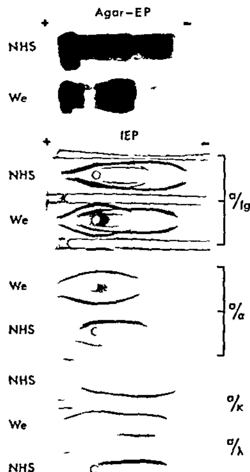


Fig 1 Agar gel electrophoresis shows the presence of a peak in the β region and decreased protein content in the γ region On immunoelectrophoresis the M-component is visible with an anti total immunoglobulin antiserum and with antisera which are specific for the α and the chain *NHS*=normal human serum *We*=patient s serum

protein was again very high A therapeutic trial as described earlier was instituted but the disease proved to be refractory the pancytopenia and the increased total protein did not respond and in addition a circulating anti coagulant was detected

The patient had to be admitted several times because of nose-bleeds Treatment with anabolic steroids did not improve the condition Recurrent infections of the respiratory tract complicated the picture and the patient expired in Nov 1969 at the age of 80 years with a clinical picture of haemorrhagic diathesis with pneumonia

At autopsy (670/69) abdominal lymph nodes and the spleen (800 g) were enlarged Microscopic examination revealed a diffuse lymphoid proliferation extending beyond praeexisting capsules in spleen lymph nodes bone marrow liver adrenals and testes The infiltrate which obscured the underlying structures consisted of mature lymphocytes small plasma cells their intermediates and of some mast cells with focal abundance of PAS positive homogeneous material between the cells (Fig 4)

The massive bone marrow infiltration was also present in the femur diaphysis and replaced almost all haematopoiesis with severe pancytopenia of the peripheral blood Bleeding tendency led to petechiae and haematomas in many sites as in both lungs Death was caused by bilateral haemorrhagic pneumonia followed by irreversible septic shock with massive erythrophagocytosis in lymphoid organs and bone marrow The diagnosis of Waldenstrom s disease could be confirmed

Special studies

Special techniques were applied for a further analysis of the combination of the clinical syndrome of Waldenstrom s disease with the presence of a κ type IgA paraprotein

Immunofluorescence This test was carried out on washed and concentrated cell suspensions with monospecific conjugates (5) The bone marrow was investigated three times and also specimens which had been obtained post mortem All samples showed an abnormally high percentage of IgA positive cells practically all of κ type In the biopsies the majority of the fluorescent cells were lymphocytes (Fig 5) but in the autopsy material there was a preponderance of fluorescent plasma cells The detailed figures are given in Table I The pattern of the fluorescent cells in the peripheral blood showed again a great excess of IgA positive cells of κ type but IgM and IgG cells were not entirely absent

Analytical ultracentrifugation Peaks with S values (uncorrected) of 4 6 6 10 9 13 and 16 9 could be distinguished with the 10 9 S peak as the major one (Fig 6)

Dimeric form Three markers which specifically recognize polymeric IgA were used by Dr J Radl (10) an antiserum that recognizes only the dimeric (polymeric) determinants of IgA an antiserum against J-chain and the ability of polymeric IgA_1 to bind the secretory component in vitro The three tests proved the presence of IgA in its

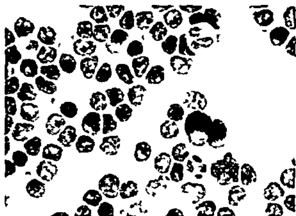


Fig 2 The bone marrow smear shows a greatly increased number of large lymphocytes and a basophilic leucocyte

Hb in gram %

14 16
12 14
10 12
8 10
6 8

platelets (x 10^4)

16 20
12 16
8 12
4-8
0-4

total protein and globulin (β+γ) content in gram %

12 14
10 12
8 10
6 8
4-6

cytostatic drugs

prednisone

1963 1964 1965 1966 1967 1968 1969

Fig 3 Overview of routine laboratory data and of scheme of treatment during the entire period of observation

polymeric (dimeric) form in the serum as well as within the plasma cells in the bone marrow as shown by immunofluorescence

Electron microscopy A purified sample of the serum IgA was examined in the electron microscope by Dr Feinstein and colleagues (2 9) The negatively stained preparation clearly showed a dimer configuration of this molecule (Fig 7)

DISCUSSION

A man sought medical advice at the age of 73 because of increasing fatigue weakness and dyspnoea on exertion No abnormalities were found at physical examination Routine laboratory investigation revealed an extremely high ESR anaemia and thrombocytopenia An M-component was found in the serum there was no Bence Jones protein and bone lesions were not detected The cytology of the bone marrow was characteristic for Waldenstrom s disease The diagnosis received support from the presence of multiple malignancies in his history from the prolonged bleeding time and from a polyneuropathy This diagnosis is also favoured by the relatively good condition during the observation of seven years and it was confirmed by the autopsy data The only unexpected finding was the presence of an IgA paraprotein instead of a macroglobulinaemia due to the presence of monoclonal IgM The IgA was of subclass 1 and of κ type The major peak on ultracentrifugation had an S value of 10 9 (uncorrected) indicating a dimer molecule The results of immunodiffusion and immunofluorescence tests which specifically distinguish the different molecular forms of IgA were also positive for the dimer configuration Direct visualization was obtained by electron microscopy Immunofluorescence showed the presence of this Ig within the large lymphocytes

In many respects the behaviour of this IgA para

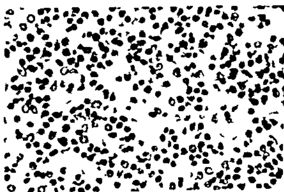


Fig 4 Section of specimen obtained post mortem

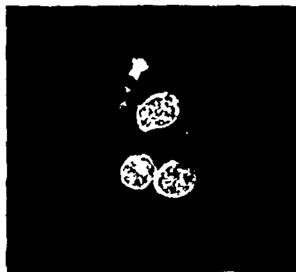


Fig 5 Immunofluorescence of bone marrow specimens shows the presence of IgA in the cytoplasm of large lymphocytes

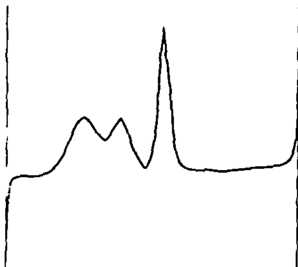


Fig 6 Ultracentrifuge pattern showing a major peak with an (uncorrected) S value of 10 9 Technical data meniscus at left 59780 rev/min 20°C

protein was remarkably similar to the behaviour of the IgM paraprotein in the classical cases of Waldenstrom s macroglobulinaemia and contrasted sharply with IgA M-components in multiple myeloma Its serum level remained relatively constant during the observation period of seven years and it only clearly increased during the last year of life Furthermore the residual normal immunoglobulins were present in a concentration to be expected in Waldenstrom s disease The percentage distribution of Ig positive cells in the bone marrow showed a preponderance of IgA-containing cells it not the near total absence of cells containing the er Ig classes as is usually the case in multiple eloma (4)

The major question however remains unanswered how to explain the development of the disorder? Any attempt to explain this malignant proliferation of large lymphocytes which produce IgA instead of IgM should take the extreme rarity of this condition into consideration This case seems to be the first on record Several authors have presented evidence that the monoclonal proliferation develops at random from the normal polyclonal population (7 8 12) On this basis one can speculate about the existence under normal conditions of a small number of lymphocytes which secrete IgA These cells would be the precursors of the IgA secreting plasma cells and be derived from IgM secretory lymphocytes which implies a switch from IgM to IgA If the existence of double positive cells can be used as an argument in favour of this idea then the pertinent observations are available the combination of IgM and IgA within one cell does exist under normal conditions The percentage of the frequency of this combination is about 0 25% which is con

Table I *Cytoplasmic immunoglobulins (%) as detected by immunofluorescence*

Date	Organ	IgA	IgM	IgG	κ	λ	Lymphocyte/plasma cell ratio
Jan 1967	Bone marrow	70	2	38	100	0	90/10
Feb 1967	Bone marrow	92	3	5	99	1	94/6
Nov 1967	Bone marrow	100	0	0	100	0	28/72
Post mortem	Spleen	100	0	0	100	0	7/93
	Lymph node	100	0	0	100	0	14/86
Normal values	Bone marrow	40	10	50	60	40	



Fig 7 Electron m crograph of the purif ed M-component shows its d mer structure (ref ? and 9 w th perm ss on of authors and publishers)

siderably lower than the other possible immuno globul n comb nations (6) The low number of b clonal gammopath es with IgM and IgA in com parison to the other b clonal gammopathy combina t ons (1) is in accordance with the concept that the number of clones becom ng mal gnant is relative to the number of d fferent clones which are present in the normal ind vidual

Nomenclature does not present a problem Waldenstrom s disease is a satisfactory name but those who prefer to adhere to the original and more mean ngful designat on of Waldenstrom s macro globulinaem a can use the argument that in this *patient too the abnormal immunoglobulin was a* macroglobulin be it of the IgA class

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Diagnosis of Cardiac Amyloidosis by Myocardial Biopsy

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ABSTRACT A case of cardiac amyloidosis with severe heart failure is presented The diagnosis was confirmed by myocardial biopsy The microscopical and ultrastructural changes in this biopsy are described in detail

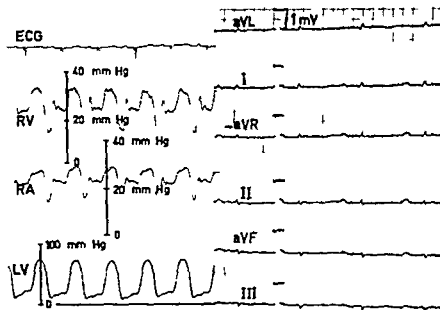
The clinical appearance of cardiac amyloidosis is usually non specific as are the results of the clinical investigation Descriptions of the pathological findings in cardiac amyloidosis (11) are based on post mortem examinations where autolytic changes can interfere to a certain degree Biopsies from the liver kidney and rectal mucosa may provide indirect evidence for the occurrence of the disease (8) Cardiac biopsy must however be considered the best method of making the diagnosis of cardiac amyloidosis This has recently been pointed out (3) but the findings of such a biopsy have as far as we know not yet been published

CASE REPORT

The patient was a man 38 years old previously in good health He was referred to hospital because of dyspnoea when exerted swelling of the ankles diarrhoea and weight reduction Upon admission slight ankle oedema was noted as well as dyspnoea upon slight physical effort Physical examination of the heart revealed no abnormal findings BP was 110/85 mmHg Routine laboratory investigations revealed an ESR of 7 mm/h a Hb concentration of 13 6 g/100 ml and WBC of 6700 The values for serum electrolytes albumin creatinine protein bound iodine and glucose were normal ECG showed sinus rhythm with a P R interval of 18 msec Apart from a conspicuously low voltage (sum of the total R S wave voltage in leads I II and III <1 5 mV) no definite pathological pattern could be recognized (Fig 1) X ray examination of the chest showed general enlargement of the heart with a total heart volume of 1090 ml corresponding to 630 ml/m² BSA Because of the h[illegible] diarrhoea and weight reduction radiological ar[illegible] logical investigations of the intestine were perfor[illegible] negative results

In view of the otherwise unexplained cl[illegible] right heart failure and the changes in the ECG amyloidosis was suspected There were no [illegible] infectious or malignant disease that might [illegible] ondary amyloidosis Tuberculine reaction w[illegible] smears from the sternal marrow 10–13 [illegible] were found with nuclear inclusion bodies s[illegible] blue with Wright s stain but apart from [illegible] was normal No skeletal destructions we[illegible] concentrations of IgG and IgA in serum were [illegible] serum immunoelectrophoresis revealed a m[illegible] clonal component of free lambda light chains de[illegible]ned by Dr G Husby Rikshospitalet Oslo Norway) [illegible] Jones protein could not be demonstrated in the u[illegible] A biopsy from the rectal mucosa showed staining properties characteristic for amyloid

To further evaluate the cardiac function the patient underwent complete right and left heart catheterization as well as an endomyocardial biopsy from the right ventricle Via a vein in the right arm a double lumen catheter no 9 was placed in the pulmonary artery wedge position with its side hole recording the pressure in the pulmonary artery A short polyethylene catheter was inserted percutaneously in the left brachial artery Finally a red Ödman Ledin catheter was inserted percutaneously in the right femoral artery and via retrograde approach the left ventricle was reached Cardiac function was evaluated at rest using routine techniques The condition of the patient did not permit any measurements during exercise The results showed signs of severe left and especially right heart failure with pulmonary artery wedge and right atrial mean pressures of almost ?0 mmHg Correspondingly the end-diastolic pressures in the left and right ventricles were of the same magnitude The pressure curve from the right ventricle was highly pathological with an early diastolic dip that was extreme followed by a late diastolic plateau This configuration of the curve was transmitted far beyond the tricuspid valves Thus the pressure curve from the superior vena cava showed an extreme ventricularization The values for cardiac index and stroke index were



F g I ECG and pressure tracings from right and left heart catheterizat on Upper left trac ng ECG lead V_4 *RV* right ventricle *RA* right atrium *LV* =left ven tricle Paper speed 25 mm/sec R ght ECG (l mb leads) paper speed 50 mm/sec

extremely low 1 ? l/m n and 15 ml/beat/m² BSA respec t vely cons stent w th a severely mpa red card ac func t on At the end of the nvest gat on endomyocard al b opsy was performed from the right ventricle us ng the same technique as described elsewhere (1?) When perform ng the b opsy t was obvious that the myocard um was very soft Only a very sl ght touch of the b opsy instrument aga nst the myocard al wall gave a b opsy specimen of adequate s ze At the moment of the b opsy no extrasystoles or other arrhythm as could be detected on the continuous ECG record ng

M croscop c exam nat on

Two b ops es from the right ventricle were taken for l ght m c oscopy They were f xed in 10% neutral formal n embedded n paraff n and stained with hematoxyl n and eos n Congo red PAS van G eson period c ac d s lver method and methyl violet Serial sect ons were prepared One b opsy from the right ventricle was prepared for electron m croscop c exam nat on The f xat on took place in 2 5% glutaraldehyde in a ch lled phosphate buffer at pH 7 3 for two hours followed by 1% osm um tetroxide in a phosphate buffer at pH 7 3 for one hour The dehydra t on was performed in an ethanol series and the tissue was contrasted w th phosphotungstic ac d passed through styrene and embedded in Vestopal Sect ons were sta ned with lead c trate and uranyl acetate In l ght m croscopy all sect ons had a s m lar appearance and showed heavy nf ltration with amylo d stain ng orange w th Congo red Exam nation n polarized l ght of Congo red stained sect ons gave green sh b refringence of the amylo d There was no metachromas a in methyl violet stained sect ons and PAS react on was negat ve



F g 2 Cross sectioned muscle cells surrounded by amy loid mantles Note empty amyloid rings Hematoxyl n and eos n ×29?

The amylo d surrounded the s ngle muscle cells l ke a mantle closely follow ng the surface (F g 2) In the contracted cells there was an accord on l ke contour of the amylo d band seem ngly ind cat ng that it followed the movement of the cell membrane In the s lver mpregna t on it was poss ble to see the brownish amylo d between the ret cular f bers and the cell surface In many places there were r ngs of amylo d w thout recogn zable muscle structures centrally g v ng the mpress on of myolys s (F g ?) In long tud nally oriented cells it was poss ble to observe an nterm ngl ng of myof brils and amylo d There was a moderate amylo d inf ltrat on of vascular walls sta n ng in the same manner as the parenchymal amylo d

Electron microscop c examinat on of the card ac b opsy d sclosed the amylo d as a band of f brillar material of even th ckness surround ng all muscle cells On the outer side of th s band collagen f brils were often seen Amyloid was also seen surround ng even the smallest vessels In many places there were no v s ble plasma membranes of the muscle cells and the amyloid seemed to be d rectly juxtaposed to intracellular structures (F g 3)



Fig 3 Amyloid mass in contact with intracellular myofibril (right) Note abrupt end of cell embedded in amyloid Approximately ×18 700

The endpoints of muscle cells were often embedded in amyloid In some places there was an elongation of sarcomeres with prominent I-banding giving the impression of stretching of the myofibrils over amyloid masses (Fig 4) In high magnification the amyloid was seen as fibrils of about 90 Å thickness with random orientation in good accordance to the findings of Roberts and Ferrans (11)

The patient returned home two weeks after the investigation His condition deteriorated slowly About four months after the investigation he died of cardiac failure Autopsy was not performed

DISCUSSION

The absence of any infectious or inflammatory disease together with the occurrence of amyloid in the heart and in the intestine as well suggested that the patient suffered from primary amyloidosis This

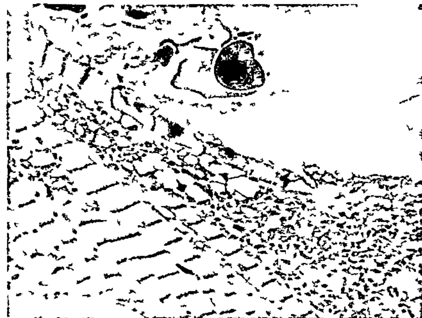


Fig 4 Cell with peripheral myofibril showing lengthened sarcomeres Approximately ×6 800

type and distribution of amyloid in younger patients may appear without any obvious etiology or associated with multiple myeloma or macroglobulinaemia (6) Our patient presented no signs of macroglobulinaemia or classical multiple myeloma On the other hand the percentage of plasma cells in the bone marrow was elevated and free monoclonal λ chains were detected in serum in low concentration A search for λ-chains in serum and urine in patients with primary amyloidosis may reveal many patients with circulating free λ chains This strengthens the connection between amyloidosis and immunopathy as suggested by Osserman et al (10) and Franklin (5) The haemodynamic findings although suggesting cardiac amyloidosis are not pathognomonic (7) In one of the cases described by Buja et al (2) and in a case reported by Crockett et al (4) thoracotomy was considered necessary to exclude constrictive pericarditis

Together with the clinical picture and the haemodynamic findings the diagnosis in our patient was established by the typical histological appearance in the myocardial biopsy where the amyloid showed positive staining and characteristic birefringence with Congo red Absence of metachromasia does not exclude amyloidosis and more than one staining method is always to be recommended in cases of suspected primary amyloidosis The ultrastructural appearance was also typical The position of the amyloid between the connective tissue fibrils and the cell surface is remarkable It conveys an impression of the amyloid being formed in close connection with the plasma membrane The intermingling of amyloid and intracellular structures could possibly be taken as a sign of intracellular formation of amyloid but we prefer to believe that the plasma membrane has been destroyed secondarily by degenerative changes e g by nutritional disturbance due to the amyloid surrounding the cell The strict adherence of the amyloid mantle to the cell surface seems to indicate that it contracted with the cell at the moment of biopsy The stretching of some sarcomeres indicates that the amyloid may be a mechanical hindrance in some positions

The soft consistency of the myocardium wh was noted during the investigation is contrast to the rubber like consistency felt at topsy We presume that amyloid at body ture is much softer than in the dead and chi body

The present case illustrates the usefulness cardiac biopsy when suspecting cardiac amyl dosis especially as the method is reliable and e to perform (1 9 12) and gives in vivo morpho cal information from the organ of primary i

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